

PATENT APPLICATION

**INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE
AND NUCLEIC ACID COMPOSITIONS**

Inventor(s): John Fikes, a United States citizen, residing at
6494 Lipmann Street
San Diego, California 92122

Alessandro Sette, an Italian citizen, residing at
5551 Linda Rosa Avenue
La Jolla, California 92037

John Sidney, a United States citizen, residing at
4218 Corte de la Siena
San Diego, California 92130

Scott Southwood, a United States citizen, residing at
10679 Strathmore Drive
Santee, California 92071

Robert Chesnut, a United States citizen, residing at
1473 Kings Cross Drive
Cardiff-by-the-Sea, California 92007

Esteban Celis, a United States citizen, residing at
3683 Wright Road S.W.
Rochester, Minnesota 55902

Elissa Keogh, a United States citizen, residing at
4343 Caminito del Diamante
San Diego, California 92121

TOWNSEND and TOWNSEND and CREW LLP
Two Embarcadero Center, 8th Floor
San Francisco, California 94111-3834
(415) 576-0200

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5 **INDUCING CELLULAR IMMUNE RESPONSES TO MAGE2/3 USING PEPTIDE
AND NUCLEIC ACID COMPOSITIONS****CROSS-REFERENCES TO RELATED APPLICATIONS**

This application is a Continuation-In-Part (“CIP”) of U.S.S.N. 09/189,702, filed 11/10/98, which is a CIP of U.S.S.N 08/205,713 filed 3/4/94, which is a CIP of abandoned U.S.S.N. 08/159,184 filed 11/29/93, which is a CIP of abandoned U.S.S.N. 08/073,205 filed 6/4/93 which is a CIP of abandoned U.S.S.N 08/027,146 filed 3/5/93. The present application is also related to U.S.S.N. 09/226,775, which is a CIP of abandoned U.S.S.N. 08/815,396, which claims benefit of abandoned U.S.S.N. 60/013,113. Furthermore, the present application is related to U.S.S.N. 09/017,735, which is a CIP of abandoned U.S.S.N. 15 08/589,108; U.S.S.N. 08/454,033; and U.S.S.N. 08/349,177. The present application is also related to U.S.S.N. 09/017,524, U.S.S.N. 08/821,739, which claims benefit of abandoned U.S.S.N. 60/013,833; and U.S.S.N. 08/347,610, which is a CIP of U.S.S.N. 08/159,339, which is a CIP of abandoned U.S.S.N. 08/103,396, which is a CIP of abandoned U.S.S.N. 08/027,746, which is a CIP of abandoned U.S.S.N. 07/926,666. The present application is 20 also related to U.S.S.N. 09/017,743, which is a CIP of abandoned U.S.S.N. 08/590,298; and U.S.S.N. 08/452,843, which is a CIP of U.S.S.N. 08/344,824, which is a CIP of abandoned U.S.S.N. 08/278,634. The present application is also related to PCT application 99/12066 filed 5/28/99 which claims benefit of provisional U.S.S.N. 60/087,192, and U.S.S.N. 09/009,953, which is a CIP of abandoned U.S.S.N. 60/036,713 and abandoned U.S.S.N. 25 60/037,432. In addition, the present application is related to U.S.S.N. 09/098,584, U.S.S.N. 09/239,043, U.S.S.N. 60/117,486, U.S.S.N. 09/350,401, and U.S.S.N. 09/357,737. In addition, the present application is related to U.S. Patent Application entitled “Inducing Cellular Immune Responses to p53 Using Peptide and Nucleic Acid Compositions”, Attorney Docket No. 018623-014500, filed of even date herewith; U.S. Patent Application 30 entitled “Inducing Cellular Immune Responses to Carcinoembryonic Antigen Using Peptide and Nucleic Acid Compositions”, Attorney Docket No. 018623-014400, filed of even date herewith; and U.S. Patent Application entitled “Inducing Cellular Immune Responses to HER2/neu Using Peptide and Nucleic Acid Compositions”, Attorney Docket No. 018623-

014800, filed of even date herewith. All of the above applications are incorporated herein by reference.

FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

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CONTINUATION SHEET

I. BACKGROUND OF THE INVENTION

A growing body of evidence suggests that cytotoxic T lymphocytes (CTL) are important in the immune response to tumor cells. CTL recognize peptide epitopes in the context of HLA class I molecules that are expressed on the surface of almost all nucleated cells. Following intracellular processing of endogenously synthesized tumor antigens, antigen-derived peptide epitopes bind to class I HLA molecules in the endoplasmic reticulum, and the resulting complex is then transported to the cell surface. CTL recognize the peptide-HLA class I complex, which then results in the destruction of the cell bearing the HLA-peptide complex directly by the CTL and/or via the activation of non-destructive mechanisms, *e.g.*, activation of lymphokines such as tumor necrosis factor- α (TNF- α) or interferon- γ (IFN γ) which enhance the immune response and facilitate the destruction of the tumor cell.

Tumor-specific helper T lymphocytes (HTLs) are also known to be important for maintaining effective antitumor immunity. Their role in antitumor immunity has been demonstrated in animal models in which these cells not only serve to provide help for induction of CTL and antibody responses, but also provide effector functions, which are mediated by direct cell contact and also by secretion of lymphokines (*e.g.*, IFN γ and TNF- α).

A fundamental challenge in the development of an efficacious tumor vaccine is immune suppression or tolerance that can occur. There is therefore a need to establish vaccine embodiments that elicit immune responses of sufficient breadth and vigor to prevent progression and/or clear the tumor.

The epitope approach, as we have described, may represent a solution to this challenge, in that it allows the incorporation of various antibody, CTL and HTL epitopes, from discrete regions of a target TAA, and/or regions of other TAAs, in a single vaccine composition. Such a composition may simultaneously target multiple dominant and subdominant epitopes and thereby be used to achieve effective immunization in a diverse population.

MAGE, melanoma antigen genes, are a family of related proteins that were first described in 1991. Van der Bruggen and co-workers identified the MAGE gene after isolating CTLs from a patient who demonstrated spontaneous tumor regression. These CTLs recognized melanoma cell lines as well as tumor lines from other patient all of whom expressed the same HLA-A1-restricted gene (van der Bruggen *et al.*, *Science*

254:1643-1647, 1991; DePlaen *et al.*, *Immunogenetics* 40:360-369, 1994). The MAGE genes are expressed in metastatic melanomas (see, e.g., Brasseur *et al.*, *Int. J. Cancer* 63:375-380, 1995), non-small lung (Weynants *et al.*, *Int. J. Cancer* 56:826-829, 1994), gastric (Inoue *et al.*, *Gastroenterology* 109:1522-1525, 1995), hepatocellular (Chen *et al.*, 5 *Liver* 19:110-114, 1999), renal (Yamanaka *et al.*, *Human Pathol.* 24:1127-1134, 1998), colorectal (Mori *et al.*, *Ann. Surg.* 224:183-188, 1996), and esophageal (Quillien *et al.*, *Anticancer Res.* 17:387-391, 1997) carcinomas as well as tumors of the head and neck (Lett *et al.*, *Acta Otolaryngol.* 116:633-639, 1996), ovaries (Gillespie *et al.*, *Br J. Cancer* 10 78:816-821, 1998; Yamada *et al.*, *Int. J. Cancer* 64:388-393, 1995), bladder, and osteosarcoma (Sudo *et al.*, *J. Orthop. Res.* 15:128-132, 1997). Thus, MAGE2/3 are important targets for cancer immunotherapy.

The information provided in this section is intended to disclose the presently understood state of the art as of the filing date of the present application. Information is included in this section which was generated subsequent to the priority date of this 15 application. Accordingly, information in this section is not intended, in any way, to delineate the priority date for the invention.

II. SUMMARY OF THE INVENTION

This invention applies our knowledge of the mechanisms by which antigen is 20 recognized by T cells, for example, to develop epitope-based vaccines directed towards TAAs. More specifically, this application communicates our discovery of specific epitope pharmaceutical compositions and methods of use in the prevention and treatment of cancer.

Upon development of appropriate technology, the use of epitope-based vaccines 25 has several advantages over current vaccines, particularly when compared to the use of whole antigens in vaccine compositions. For example, immunosuppressive epitopes that may be present in whole antigens can be avoided with the use of epitope-based vaccines. Such immunosuppressive epitopes may, e.g., correspond to immunodominant epitopes in whole antigens, which may be avoided by selecting peptide epitopes from non-dominant 30 regions (see, e.g., Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996).

An additional advantage of an epitope-based vaccine approach is the ability to combine selected epitopes (CTL and HTL), and further, to modify the composition of the epitopes, achieving, for example, enhanced immunogenicity. Accordingly, the immune

DETAILED DESCRIPTION

response can be modulated, as appropriate, for the target disease. Similar engineering of the response is not possible with traditional approaches.

Another major benefit of epitope-based immune-stimulating vaccines is their safety. The possible pathological side effects caused by infectious agents or whole

5 protein antigens, which might have their own intrinsic biological activity, is eliminated.

An epitope-based vaccine also provides the ability to direct and focus an immune response to multiple selected antigens from the same pathogen (a “pathogen” may be an infectious agent or a tumor associated molecule). Thus, patient-by-patient variability in the immune response to a particular pathogen may be alleviated by inclusion of epitopes
10 from multiple antigens from the pathogen in a vaccine composition.

Furthermore, an epitope-based anti-tumor vaccine also provides the opportunity to combine epitopes derived from multiple tumor-associated molecules. This capability can therefore address the problem of tumor-to tumor variability that arises when developing a broadly targeted anti-tumor vaccine for a given tumor type and can also reduce the
15 likelihood of tumor escape due to antigen loss. For example, a melanoma in one patient may express a target TAA that differs from a melanoma in another patient. Epitopes derived from multiple TAAs can be included in a polyepitopic vaccine that will target both melanomas.

One of the most formidable obstacles to the development of broadly efficacious
20 epitope-based immunotherapeutics, however, has been the extreme polymorphism of HLA molecules. To date, effective non-genetically biased coverage of a population has been a task of considerable complexity; such coverage has required that epitopes be used that are specific for HLA molecules corresponding to each individual HLA allele.

Impractically large numbers of epitopes would therefore have to be used in order to cover
25 ethnically diverse populations. Thus, there has existed a need for peptide epitopes that are bound by multiple HLA antigen molecules for use in epitope-based vaccines. The greater the number of HLA antigen molecules bound, the greater the breadth of population coverage by the vaccine.

Furthermore, as described herein in greater detail, a need has existed to modulate
30 peptide binding properties, *e.g.*, so that peptides that are able to bind to multiple HLA molecules do so with an affinity that will stimulate an immune response. Identification of epitopes restricted by more than one HLA allele at an affinity that correlates with immunogenicity is important to provide thorough population coverage, and to allow the elicitation of responses of sufficient vigor to prevent or clear an infection in a diverse

segment of the population. Such a response can also target a broad array of epitopes. The technology disclosed herein provides for such favored immune responses.

In a preferred embodiment, epitopes for inclusion in vaccine compositions of the invention are selected by a process whereby protein sequences of known antigens are evaluated for the presence of motif or supermotif-bearing epitopes. Peptides

5 corresponding to a motif- or supermotif-bearing epitope are then synthesized and tested for the ability to bind to the HLA molecule that recognizes the selected motif. Those peptides that bind at an intermediate or high affinity *i.e.*, an IC₅₀ (or a K_D value) of 500 nM or less for HLA class I molecules or an IC₅₀ of 1000 nM or less for HLA class II molecules, are further evaluated for their ability to induce a CTL or HTL response.

10 Immunogenic peptide epitopes are selected for inclusion in vaccine compositions.

15 Supermotif-bearing peptides may additionally be tested for the ability to bind to multiple alleles within the HLA supertype family. Moreover, peptide epitopes may be analogued to modify binding affinity and/or the ability to bind to multiple alleles within an HLA supertype.

The invention also includes embodiments comprising methods for monitoring or evaluating an immune response to a TAA in a patient having a known HLA-type. Such methods comprise incubating a T lymphocyte sample from the patient with a peptide composition comprising a TAA epitope that has an amino acid sequence described in Tables VII to Table XX or Table XXII which binds the product of at least one HLA allele present in the patient, and detecting for the presence of a T lymphocyte that binds to the peptide. A CTL peptide epitope may, for example, be used as a component of a tetrameric complex for this type of analysis.

An alternative modality for defining the peptide epitopes in accordance with the invention is to recite the physical properties, such as length; primary structure; or charge, which are correlated with binding to a particular allele-specific HLA molecule or group of allele-specific HLA molecules. A further modality for defining peptide epitopes is to recite the physical properties of an HLA binding pocket, or properties shared by several allele-specific HLA binding pockets (*e.g.* pocket configuration and charge distribution) and reciting that the peptide epitope fits and binds to the pocket or pockets.

As will be apparent from the discussion below, other methods and embodiments are also contemplated. Further, novel synthetic peptides produced by any of the methods described herein are also part of the invention.

III. BRIEF DESCRIPTION OF THE FIGURES

not applicable

IV. DETAILED DESCRIPTION OF THE INVENTION

5 The peptide epitopes and corresponding nucleic acid compositions of the present invention are useful for stimulating an immune response to a TAA by stimulating the production of CTL or HTL responses. The peptide epitopes, which are derived directly or indirectly from native TAA protein amino acid sequences, are able to bind to HLA molecules and stimulate an immune response to the TAA. The complete sequence of the
10 TAA proteins to be analyzed can be obtained from GenBank. Peptide epitopes and analogs thereof can also be readily determined from sequence information that may subsequently be discovered for heretofore unknown variants of particular TAAs, as will be clear from the disclosure provided below.

A list of target TAA includes, but is not limited to, the following antigens: MAGE
15 1, MAGE 2, MAGE 3, MAGE-11, MAGE-A10, BAGE, GAGE, RAGE, MAGE-C1,
 LAGE-1, CAG-3, DAM, MUC1, MUC2, MUC18, NY-ESO-1, MUM-1, CDK4, BRCA2,
 NY-LU-1, NY-LU-7, NY-LU-12, CASP8, RAS, KIAA-2-5, SCCs, p53, p73, CEA, Her
 2/neu, Melan-A, gp100, tyrosinase, TRP2, gp75/TRP1, kallikrein, PSM, PAP, PSA, PT1-
 1, B-catenin, PRAME, Telomerase, FAK, cyclin D1 protein, NOEY2, EGF-R, SART-1,
20 CAPB, HPVE7, p15, Folate receptor CDC27, PAGE-1, and PAGE-4.

The peptide epitopes of the invention have been identified in a number of ways, as will be discussed below. Also discussed in greater detail is that analog peptides have been derived and the binding activity for HLA molecules modulated by modifying specific amino acid residues to create peptide analogs exhibiting altered immunogenicity.
25 Further, the present invention provides compositions and combinations of compositions that enable epitope-based vaccines that are capable of interacting with HLA molecules encoded by various genetic alleles to provide broader population coverage than prior vaccines.

30 IV.A. Definitions

The invention can be better understood with reference to the following definitions, which are listed alphabetically:

A “computer” or “computer system” generally includes: a processor; at least one information storage/retrieval apparatus such as, for example, a hard drive, a disk drive or

a tape drive; at least one input apparatus such as, for example, a keyboard, a mouse, a touch screen, or a microphone; and display structure. Additionally, the computer may include a communication channel in communication with a network. Such a computer may include more or less than what is listed above.

- 5 "Cross-reactive binding" indicates that a peptide is bound by more than one HLA molecule; a synonym is degenerate binding.

A "cryptic epitope" elicits a response by immunization with an isolated peptide, but the response is not cross-reactive *in vitro* when intact whole protein which comprises the epitope is used as an antigen.

- 10 A "dominant epitope" is an epitope that induces an immune response upon immunization with a whole native antigen (see, e.g., Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993). Such a response is cross-reactive *in vitro* with an isolated peptide epitope.

- 15 With regard to a particular amino acid sequence, an "epitope" is a set of amino acid residues which is involved in recognition by a particular immunoglobulin, or in the context of T cells, those residues necessary for recognition by T cell receptor proteins and/or Major Histocompatibility Complex (MHC) receptors. In an immune system setting, *in vivo* or *in vitro*, an epitope is the collective features of a molecule, such as primary, secondary and tertiary peptide structure, and charge, that together form a site
20 recognized by an immunoglobulin, T cell receptor or HLA molecule. Throughout this disclosure epitope and peptide are often used interchangeably. It is to be appreciated, however, that isolated or purified protein or peptide molecules larger than and comprising an epitope of the invention are still within the bounds of the invention.

- 25 "Human Leukocyte Antigen" or "HLA" is a human class I or class II Major Histocompatibility Complex (MHC) protein (see, e.g., Stites, *et al.*, *IMMUNOLOGY*, 8TH ED., Lange Publishing, Los Altos, CA, 1994).

- 30 An "HLA supertype or family", as used herein, describes sets of HLA molecules grouped on the basis of shared peptide-binding specificities. HLA class I molecules that share somewhat similar binding affinity for peptides bearing certain amino acid motifs are grouped into HLA supertypes. The terms HLA superfamily, HLA supertype family, HLA family, and HLA xx-like molecules (where xx denotes a particular HLA type), are synonyms.

Throughout this disclosure, results are expressed in terms of "IC₅₀'s." IC₅₀ is the concentration of peptide in a binding assay at which 50% inhibition of binding of a

reference peptide is observed. Given the conditions in which the assays are run (*i.e.*, limiting HLA proteins and labeled peptide concentrations), these values approximate K_D values. Assays for determining binding are described in detail, *e.g.*, in PCT publications WO 94/20127 and WO 94/03205. It should be noted that IC_{50} values can change, often 5 dramatically, if the assay conditions are varied, and depending on the particular reagents used (*e.g.*, HLA preparation, *etc.*). For example, excessive concentrations of HLA molecules will increase the apparent measured IC_{50} of a given ligand.

Alternatively, binding is expressed relative to a reference peptide. Although as a particular assay becomes more, or less, sensitive, the IC_{50} 's of the peptides tested may 10 change somewhat, the binding relative to the reference peptide will not significantly change. For example, in an assay run under conditions such that the IC_{50} of the reference peptide increases 10-fold, the IC_{50} values of the test peptides will also shift approximately 15 10-fold. Therefore, to avoid ambiguities, the assessment of whether a peptide is a good, intermediate, weak, or negative binder is generally based on its IC_{50} , relative to the IC_{50} of a standard peptide.

Binding may also be determined using other assay systems including those using: live cells (*e.g.*, Ceppellini *et al.*, *Nature* 339:392, 1989; Christnick *et al.*, *Nature* 352:67, 1991; Busch *et al.*, *Int. Immunol.* 2:443, 1990; Hill *et al.*, *J. Immunol.* 147:189, 1991; del Guercio *et al.*, *J. Immunol.* 154:685, 1995), cell free systems using detergent lysates (*e.g.*, Cerundolo *et al.*, *J. Immunol.* 21:2069, 1991), immobilized purified MHC (*e.g.*, Hill *et al.*, *J. Immunol.* 152, 2890, 1994; Marshall *et al.*, *J. Immunol.* 152:4946, 1994), ELISA 20 systems (*e.g.*, Reay *et al.*, *EMBO J.* 11:2829, 1992), surface plasmon resonance (*e.g.*, Khilko *et al.*, *J. Biol. Chem.* 268:15425, 1993); high flux soluble phase assays (Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994), and measurement of class I MHC stabilization or 25 assembly (*e.g.*, Ljunggren *et al.*, *Nature* 346:476, 1990; Schumacher *et al.*, *Cell* 62:563, 1990; Townsend *et al.*, *Cell* 62:285, 1990; Parker *et al.*, *J. Immunol.* 149:1896, 1992).

As used herein, "high affinity" with respect to HLA class I molecules is defined as binding with an IC_{50} , or K_D value, of 50 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 50 and about 500 nM. "High affinity" with 30 respect to binding to HLA class II molecules is defined as binding with an IC_{50} or K_D value of 100 nM or less; "intermediate affinity" is binding with an IC_{50} or K_D value of between about 100 and about 1000 nM.

The terms "identical" or percent "identity," in the context of two or more peptide sequences, refer to two or more sequences or subsequences that are the same or have a

specified percentage of amino acid residues that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using a sequence comparison algorithm or by manual alignment and visual inspection.

An "immunogenic peptide" or "peptide epitope" is a peptide that comprises an allele-specific motif or supermotif such that the peptide will bind an HLA molecule and induce a CTL and/or HTL response. Thus, immunogenic peptides of the invention are capable of binding to an appropriate HLA molecule and thereafter inducing a cytotoxic T cell response, or a helper T cell response, to the antigen from which the immunogenic peptide is derived.

The phrases "isolated" or "biologically pure" refer to material which is substantially or essentially free from components which normally accompany the material as it is found in its native state. Thus, isolated peptides in accordance with the invention preferably do not contain materials normally associated with the peptides in their *in situ* environment.

"Major Histocompatibility Complex" or "MHC" is a cluster of genes that plays a role in control of the cellular interactions responsible for physiologic immune responses. In humans, the MHC complex is also known as the HLA complex. For a detailed description of the MHC and HLA complexes, see, Paul, FUNDAMENTAL IMMUNOLOGY, 3RD ED., Raven Press, New York, 1993.

The term "motif" refers to the pattern of residues in a peptide of defined length, usually a peptide of from about 8 to about 13 amino acids for a class I HLA motif and from about 6 to about 25 amino acids for a class II HLA motif, which is recognized by a particular HLA molecule. Peptide motifs are typically different for each protein encoded by each human HLA allele and differ in the pattern of the primary and secondary anchor residues.

A "negative binding residue" or "deleterious residue" is an amino acid which, if present at certain positions (typically not primary anchor positions) in a peptide epitope, results in decreased binding affinity of the peptide for the peptide's corresponding HLA molecule.

The term "peptide" is used interchangeably with "oligopeptide" in the present specification to designate a series of residues, typically L-amino acids, connected one to the other, typically by peptide bonds between the α -amino and carboxyl groups of adjacent amino acids. The preferred CTL-inducing peptides of the invention are 13 residues or less in length and usually consist of between about 8 and about 11 residues,

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preferably 9 or 10 residues. The preferred HTL-inducing oligopeptides are less than about 50 residues in length and usually consist of between about 6 and about 30 residues, more usually between about 12 and 25, and often between about 15 and 20 residues.

5 "Pharmaceutically acceptable" refers to a non-toxic, inert, and/or physiologically compatible composition.

A "primary anchor residue" is an amino acid at a specific position along a peptide sequence which is understood to provide a contact point between the immunogenic peptide and the HLA molecule. One to three, usually two, primary anchor residues within a peptide of defined length generally defines a "motif" for an immunogenic 10 peptide. These residues are understood to fit in close contact with peptide binding grooves of an HLA molecule, with their side chains buried in specific pockets of the binding grooves themselves. In one embodiment, for example, the primary anchor residues are located at position 2 (from the amino terminal position) and at the carboxyl 15 terminal position of a 9-residue peptide epitope in accordance with the invention. The primary anchor positions for each motif and supermotif are set forth in Table 1. For example, analog peptides can be created by altering the presence or absence of particular residues in these primary anchor positions. Such analogs are used to modulate the binding affinity of a peptide comprising a particular motif or supermotif.

20 "Promiscuous recognition" is where a distinct peptide is recognized by the same T cell clone in the context of various HLA molecules. Promiscuous recognition or binding is synonymous with cross-reactive binding.

25 A "protective immune response" or "therapeutic immune response" refers to a CTL and/or an HTL response to an antigen derived from an infectious agent or a tumor antigen, which prevents or at least partially arrests disease symptoms or progression. The immune response may also include an antibody response which has been facilitated by the stimulation of helper T cells.

The term "residue" refers to an amino acid or amino acid mimetic incorporated into an oligopeptide by an amide bond or amide bond mimetic.

30 A "secondary anchor residue" is an amino acid at a position other than a primary anchor position in a peptide which may influence peptide binding. A secondary anchor residue occurs at a significantly higher frequency amongst bound peptides than would be expected by random distribution of amino acids at one position. The secondary anchor residues are said to occur at "secondary anchor positions." A secondary anchor residue can be identified as a residue which is present at a higher frequency among high or

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intermediate affinity binding peptides, or a residue otherwise associated with high or intermediate affinity binding. For example, analog peptides can be created by altering the presence or absence of particular residues in these secondary anchor positions. Such analogs are used to finely modulate the binding affinity of a peptide comprising a
5 particular motif or supermotif.

A "subdominant epitope" is an epitope which evokes little or no response upon immunization with whole antigens which comprise the epitope, but for which a response can be obtained by immunization with an isolated peptide, and this response (unlike the case of cryptic epitopes) is detected when whole protein is used to recall the response *in*
10 *vitro* or *in vivo*.

A "supermotif" is a peptide binding specificity shared by HLA molecules encoded by two or more HLA alleles. Preferably, a supermotif-bearing peptide is recognized with high or intermediate affinity (as defined herein) by two or more HLA molecules.

"Synthetic peptide" refers to a peptide that is not obtained from natural sources,
15 but is man-made using such methods as chemical synthesis or recombinant DNA technology.

The nomenclature used to describe peptide compounds follows the conventional practice wherein the amino group is presented to the left (the N-terminus) and the carboxyl group to the right (the C-terminus) of each amino acid residue. When amino acid residue positions are referred to in a peptide epitope they are numbered in an amino
20 to carboxyl direction with position one being the position closest to the amino terminal end of the epitope, or the peptide or protein of which it may be a part. In the formulae representing selected specific embodiments of the present invention, the amino- and carboxyl-terminal groups, although not specifically shown, are in the form they would
25 assume at physiologic pH values, unless otherwise specified. In the amino acid structure formulae, each residue is generally represented by standard three letter or single letter designations. The L-form of an amino acid residue is represented by a capital single letter or a capital first letter of a three-letter symbol, and the D-form for those amino acids having D-forms is represented by a lower case single letter or a lower case three letter symbol. Glycine has no asymmetric carbon atom and is simply referred to as "Gly" or G.
30 Symbols for the amino acids are shown below.

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Single Letter Symbol	Three Letter Symbol	Amino Acids
A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic Acid
E	Glu	Glutamic Acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

IV.B. Stimulation of CTL and HTL responses

The mechanism by which T cells recognize antigens has been delineated during
5 the past ten years. Based on our understanding of the immune system we have developed efficacious peptide epitope vaccine compositions that can induce a therapeutic or prophylactic immune response to a TAA in a broad population. For an understanding of the value and efficacy of the claimed compositions, a brief review of immunology-related technology is provided.

10 A complex of an HLA molecule and a peptidic antigen acts as the ligand recognized by HLA-restricted T cells (Buus, S. *et al.*, *Cell* 47:1071, 1986; Babbitt, B. P. *et al.*, *Nature* 317:359, 1985; Townsend, A. and Bodmer, H., *Annu. Rev. Immunol.* 7:601,

1989; Germain, R. N., *Annu. Rev. Immunol.* 11:403, 1993). Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues that correspond to motifs required for specific binding to HLA antigen molecules have been identified and are described herein
 5 and are set forth in Tables I, II, and III (see also, e.g., Southwood, *et al.*, *J. Immunol.* 160:3363, 1998; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995; Rammensee *et al.*, SYFPEITHI, access via web at : <http://134.2.96.221/scripts.hlaserver.dll/home.htm>; Sette, A. and Sidney, *J. Curr. Opin. Immunol.* 10:478, 1998; Engelhard, V. H., *Curr. Opin. Immunol.* 6:13, 1994; Sette, A. and Grey, H. M., *Curr. Opin. Immunol.* 4:79, 1992;
 10 Sinigaglia, F. and Hammer, J. *Curr. Biol.* 6:52, 1994; Ruppert *et al.*, *Cell* 74:929-937, 1993; Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; Sidney *et al.*, *J. Immunol.* 157:3480-3490, 1996; Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; Sette, A. and Sidney, *J. Immunogenetics*, in press, 1999).

Furthermore, x-ray crystallographic analysis of HLA-peptide complexes has
 15 revealed pockets within the peptide binding cleft of HLA molecules which accommodate, in an allele-specific mode, residues borne by peptide ligands; these residues in turn determine the HLA binding capacity of the peptides in which they are present. (See, e.g., Madden, D.R. *Annu. Rev. Immunol.* 13:587, 1995; Smith, *et al.*, *Immunity* 4:203, 1996; Fremont *et al.*, *Immunity* 8:305, 1998; Stern *et al.*, *Structure* 2:245, 1994; Jones, E.Y.
 20 *Curr. Opin. Immunol.* 9:75, 1997; Brown, J. H. *et al.*, *Nature* 364:33, 1993; Guo, H. C. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:8053, 1993; Guo, H. C. *et al.*, *Nature* 360:364, 1992; Silver, M. L. *et al.*, *Nature* 360:367, 1992; Matsumura, M. *et al.*, *Science* 257:927, 1992; Madden *et al.*, *Cell* 70:1035, 1992; Fremont, D. H. *et al.*, *Science* 257:919, 1992; Saper, M. A. , Bjorkman, P. J. and Wiley, D. C., *J. Mol. Biol.* 219:277, 1991.)

25 Accordingly, the definition of class I and class II allele-specific HLA binding motifs, or class I or class II supermotifs allows identification of regions within a protein that have the potential of binding particular HLA molecules.

The present inventors have found that the correlation of binding affinity with immunogenicity, which is disclosed herein, is an important factor to be considered when
 30 evaluating candidate peptides. Thus, by a combination of motif searches and HLA-peptide binding assays, candidates for epitope-based vaccines have been identified. After determining their binding affinity, additional confirmatory work can be performed to select, amongst these vaccine candidates, epitopes with preferred characteristics in terms of population coverage, antigenicity, and immunogenicity.

Various strategies can be utilized to evaluate immunogenicity, including:

1) Evaluation of primary T cell cultures from normal individuals (see, e.g.,

Wentworth, P. A. *et al.*, *Mol. Immunol.* 32:603, 1995; Celis, E. *et al.*, *Proc. Natl. Acad. Sci. USA* 91:2105, 1994; Tsai, V. *et al.*, *J. Immunol.* 158:1796, 1997; Kawashima, I. *et al.*, *Human Immunol.* 59:1, 1998);

5 This procedure involves the stimulation of peripheral blood lymphocytes (PBL) from normal subjects with a test peptide in the presence of antigen presenting cells *in vitro* over a period of several weeks. T cells specific for the peptide become activated during this time and are detected using, e.g., a ^{51}Cr -release assay involving peptide sensitized target cells.

10 2) Immunization of HLA transgenic mice (see, e.g., Wentworth, P. A. *et al.*, *J. Immunol.* 26:97, 1996; Wentworth, P. A. *et al.*, *Int. Immunol.* 8:651, 1996; Alexander, J. *et al.*, *J. Immunol.* 159:4753, 1997); In this method, peptides in incomplete Freund's adjuvant are administered subcutaneously to HLA transgenic mice. Several weeks following immunization, splenocytes are removed and cultured *in vitro* in the presence of test peptide for approximately one week. Peptide-specific T cells are detected using, e.g., a ^{51}Cr -release assay involving peptide sensitized target cells and target cells expressing endogenously generated antigen.

15 3) Demonstration of recall T cell responses from patients who have been effectively vaccinated or who have a tumor; (see, e.g., Rehermann, B. *et al.*, *J. Exp. Med.* 181:1047, 1995; Doolan, D. L. *et al.*, *Immunity* 7:97, 1997; Bertoni, R. *et al.*, *J. Clin. Invest.* 100:503, 1997; Threlkeld, S. C. *et al.*, *J. Immunol.* 159:1648, 1997; Diepolder, H. M. *et al.*, *J. Virol.* 71:6011, 1997; Tsang *et al.*, *J. Natl. Cancer Inst.* 87:982-990, 1995; Disis *et al.*, *J. Immunol.* 156:3151-3158, 1996). In applying this strategy, recall responses are detected by culturing PBL from patients with cancer who have generated an immune response "naturally", or from patients who were vaccinated with tumor antigen vaccines. PBL from subjects are cultured *in vitro* for 1-2 weeks in the presence of test peptide plus antigen presenting cells (APC) to allow activation of "memory" T cells, as compared to "naive" T cells. At the end of the culture period, T cell activity is detected using assays for T cell activity including ^{51}Cr release involving peptide-sensitized targets, T cell proliferation, or lymphokine release.

20 The following describes the peptide epitopes and corresponding nucleic acids of the invention.

IV.C. Binding Affinity of Peptide Epitopes for HLA Molecules

As indicated herein, the large degree of HLA polymorphism is an important factor to be taken into account with the epitope-based approach to vaccine development. To address this factor, epitope selection encompassing identification of peptides capable of binding at high or intermediate affinity to multiple HLA molecules is preferably utilized, most preferably these epitopes bind at high or intermediate affinity to two or more allele-specific HLA molecules.

CTL-inducing peptides of interest for vaccine compositions preferably include those that have an IC₅₀ or binding affinity value for class I HLA molecules of 500 nM or better (*i.e.*, the value is ≤ 500 nM). HTL-inducing peptides preferably include those that have an IC₅₀ or binding affinity value for class II HLA molecules of 1000 nM or better, (*i.e.*, the value is ≤ 1,000 nM). For example, peptide binding is assessed by testing the capacity of a candidate peptide to bind to a purified HLA molecule *in vitro*. Peptides exhibiting high or intermediate affinity are then considered for further analysis. Selected peptides are tested on other members of the supertype family. In preferred embodiments, peptides that exhibit cross-reactive binding are then used in cellular screening analyses or vaccines.

As disclosed herein, higher HLA binding affinity is correlated with greater immunogenicity. Greater immunogenicity can be manifested in several different ways. Immunogenicity corresponds to whether an immune response is elicited at all, and to the vigor of any particular response, as well as to the extent of a population in which a response is elicited. For example, a peptide might elicit an immune response in a diverse array of the population, yet in no instance produce a vigorous response. Moreover, higher binding affinity peptides lead to more vigorous immunogenic responses. As a result, less peptide is required to elicit a similar biological effect if a high or intermediate affinity binding peptide is used. Thus, in preferred embodiments of the invention, high or intermediate affinity binding epitopes are particularly useful.

The relationship between binding affinity for HLA class I molecules and immunogenicity of discrete peptide epitopes on bound antigens has been determined for the first time in the art by the present inventors. The correlation between binding affinity and immunogenicity was analyzed in two different experimental approaches (*see, e.g.*, Sette, *et al.*, *J. Immunol.* 153:5586-5592, 1994). In the first approach, the immunogenicity of potential epitopes ranging in HLA binding affinity over a 10,000-fold

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range was analyzed in HLA-A*0201 transgenic mice. In the second approach, the antigenicity of approximately 100 different hepatitis B virus (HBV)-derived potential epitopes, all carrying A*0201 binding motifs, was assessed by using PBL from acute hepatitis patients. Pursuant to these approaches, it was determined that an affinity 5 threshold value of approximately 500 nM (preferably 50 nM or less) determines the capacity of a peptide epitope to elicit a CTL response. These data are true for class I binding affinity measurements for naturally processed peptides and for synthesized T cell epitopes. These data also indicate the important role of determinant selection in the shaping of T cell responses (*see, e.g.*, Schaeffer *et al.*, *Proc. Natl. Acad. Sci. USA* 10 86:4649-4653, 1989).

An affinity threshold associated with immunogenicity in the context of HLA class II DR molecules has also been delineated (*see, e.g.*, Southwood *et al.* *J. Immunology* 160:3363-3373, 1998, and co-pending U.S.S.N. 09/009,953 filed 1/21/98). In order to define a biologically significant threshold of DR binding affinity, a database of the 15 binding affinities of 32 DR-restricted epitopes for their restricting element (*i.e.*, the HLA molecule that binds the motif) was compiled. In approximately half of the cases (15 of 32 epitopes), DR restriction was associated with high binding affinities, *i.e.* binding affinity values of 100 nM or less. In the other half of the cases (16 of 32), DR restriction was associated with intermediate affinity (binding affinity values in the 100-1000 nM range). 20 In only one of 32 cases was DR restriction associated with an IC₅₀ of 1000 nM or greater. Thus, 1000 nM can be defined as an affinity threshold associated with immunogenicity in the context of DR molecules.

In the case of tumor-associated antigens, many CTL peptide epitopes that have been shown to induce CTL that lyse peptide-pulsed target cells and tumor cell targets 25 endogenously expressing the epitope exhibit binding affinity or IC₅₀ values of 200 nM or less. In a study that evaluated the association of binding affinity and immunogenicity of such TAA epitopes, 100% (10/10) of the high binders, *i.e.*, peptide epitopes binding at an affinity of 50 nM or less, were immunogenic and 80% (8/10) of them elicited CTLs that specifically recognized tumor cells. In the 51 to 200 nM range, very similar figures were 30 obtained. CTL inductions positive for peptide and tumor cells were noted for 86% (6/7) and 71% (5/7) of the peptides, respectively. In the 201-500 nM range, most peptides (4/5 wildtype) were positive for induction of CTL recognizing wildtype peptide, but tumor recognition was not detected.

The binding affinity of peptides for HLA molecules can be determined as described in Example 1, below.

IV.D. Peptide Epitope Binding Motifs and Supermotifs

5 Through the study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides, critical residues required for allele-specific binding to HLA molecules have been identified. The presence of these residues correlates with binding affinity for HLA molecules. The identification of motifs and/or supermotifs that correlate with high and intermediate affinity binding is
10 an important issue with respect to the identification of immunogenic peptide epitopes for the inclusion in a vaccine. Kast *et al.* (*J. Immunol.* 152:3904-3912, 1994) have shown that motif-bearing peptides account for 90% of the epitopes that bind to allele-specific HLA class I molecules. In this study all possible peptides of 9 amino acids in length and overlapping by eight amino acids (240 peptides), which cover the entire sequence of the
15 E6 and E7 proteins of human papillomavirus type 16, were evaluated for binding to five allele-specific HLA molecules that are expressed at high frequency among different ethnic groups. This unbiased set of peptides allowed an evaluation of the predictive value of HLA class I motifs. From the set of 240 peptides, 22 peptides were identified that bound to an allele-specific HLA molecule with high or intermediate affinity. Of these 22
20 peptides, 20 (*i.e.* 91%) were motif-bearing. Thus, this study demonstrates the value of motifs for the identification of peptide epitopes for inclusion in a vaccine: application of motif-based identification techniques will identify about 90% of the potential epitopes in a target antigen protein sequence.

Such peptide epitopes are identified in the Tables described below.

25 Peptides of the present invention may also comprise epitopes that bind to MHC class II DR molecules. A greater degree of heterogeneity in both size and binding frame position of the motif, relative to the N and C termini of the peptide, exists for class II peptide ligands. This increased heterogeneity of HLA class II peptide ligands is due to the structure of the binding groove of the HLA class II molecule which, unlike its class I counterpart, is open at both ends. Crystallographic analysis of HLA class II DRB*0101-peptide complexes showed that the major energy of binding is contributed by peptide residues complexed with complementary pockets on the DRB*0101 molecules. An important anchor residue engages the deepest hydrophobic pocket (*see, e.g.*, Madden, D.R. *Ann. Rev. Immunol.* 13:587, 1995) and is referred to as position 1 (P1). P1 may

represent the N-terminal residue of a class II binding peptide epitope, but more typically is flanked towards the N-terminus by one or more residues. Other studies have also pointed to an important role for the peptide residue in the 6th position towards the C-terminus, relative to P1, for binding to various DR molecules.

5 In the past few years evidence has accumulated to demonstrate that a large fraction of HLA class I and class II molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires, and consensus structures of the main peptide binding pockets. Thus, peptides of the present invention are identified by any one of several HLA-specific amino acid motifs (*see, e.g.,* 10 Tables I-III), or if the presence of the motif corresponds to the ability to bind several allele-specific HLA molecules, a supermotif. The HLA molecules that bind to peptides that possess a particular amino acid supermotif are collectively referred to as an HLA “supertype.”

15 The peptide motifs and supermotifs described below, and summarized in Tables I-III, provide guidance for the identification and use of peptide epitopes in accordance with the invention.

20 Examples of peptide epitopes bearing a respective supermotif or motif are included in Tables as designated in the description of each motif or supermotif below. The Tables include a binding affinity ratio listing for some of the peptide epitopes. The ratio may be converted to IC₅₀ by using the following formula: IC₅₀ of the standard peptide/ratio = IC₅₀ of the test peptide (*i.e.*, the peptide epitope). The IC₅₀ values of standard peptides used to determine binding affinities for Class I peptides are shown in Table IV. The IC₅₀ values of standard peptides used to determine binding affinities for Class II peptides are shown in Table V. The peptides used as standards for the binding 25 assays described herein are examples of standards; alternative standard peptides can also be used when performing binding studies.

30 To obtain the peptide epitope sequences listed in each Table, protein sequence data for MAGE2/3 were evaluated for the presence of the designated supermotif or motif. The “pos” (position) column in the Tables designates the amino acid position in the MAGE2/3 proteins that corresponds to the first amino acid residue of the putative epitope. The “number of amino acids” indicates the number of residues in the epitope sequence. Preferred epitopes are those that are conserved between the MAGE2 and MAGE3 sequences. The “A” and “B” designations on the Tables refer to MAGE2 and MAGE3, respectively.

HLA Class I Motifs Indicative of CTL Inducing Peptide Epitopes:

The primary anchor residues of the HLA class I peptide epitope supermotifs and motifs delineated below are summarized in Table I. The HLA class I motifs set out in Table I(a) are those most particularly relevant to the invention claimed here. Primary and secondary anchor positions are summarized in Table II. Allele-specific HLA molecules that comprise HLA class I supertype families are listed in Table VI. In some cases, peptide epitopes may be listed in both a motif and a supermotif Table. The relationship of a particular motif and respective supermotif is indicated in the description of the individual motifs.

IV.D.1. HLA-A1 supermotif

The HLA-A1 supermotif is characterized by the presence in peptide ligands of a small (T or S) or hydrophobic (L, I, V, or M) primary anchor residue in position 2, and an aromatic (Y, F, or W) primary anchor residue at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind to the A1 supermotif (*i.e.*, the HLA-A1 supertype) is comprised of at least: A*0101, A*2601, A*2602, A*2501, and A*3201 (*see, e.g.*, DiBrino, M. *et al.*, *J. Immunol.* 151:5930, 1993; DiBrino, M. *et al.*, *J. Immunol.* 152:620, 1994; Kondo, A. *et al.*, *Immunogenetics* 45:249, 1997). Other allele-specific HLA molecules predicted to be members of the A1 superfamily are shown in Table VI. Peptides binding to each of the individual HLA proteins can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A1 supermotif are set forth on the attached Table VII.

IV.D.2. HLA-A2 supermotif

Primary anchor specificities for allele-specific HLA-A2.1 molecules (*see, e.g.*, Falk *et al.*, *Nature* 351:290-296, 1991; Hunt *et al.*, *Science* 255:1261-1263, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992; Ruppert *et al.*, *Cell* 74:929-937, 1993) and cross-reactive binding among HLA-A2 and -A28 molecules have been described. (*See, e.g.*, Fruci *et al.*, *Human Immunol.* 38:187-192, 1993; Tanigaki *et al.*, *Human Immunol.* 39:155-162, 1994; Del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994 for reviews of relevant data.) These primary anchor

residues define the HLA-A2 supermotif; which presence in peptide ligands corresponds to the ability to bind several different HLA-A2 and -A28 molecules. The HLA-A2 supermotif comprises peptide ligands with L, I, V, M, A, T, or Q as a primary anchor residue at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope.

The corresponding family of HLA molecules (*i.e.*, the HLA-A2 supertype that binds these peptides) is comprised of at least: A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, and A*6901. Other allele-specific HLA molecules predicted to be members of the A2 superfamily are shown in Table VI. As explained in detail below, binding to each of the individual allele-specific HLA molecules can be modulated by substitutions at the primary anchor and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise an A2 supermotif are set forth on the attached Table VIII. The motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.3. HLA-A3 supermotif

The HLA-A3 supermotif is characterized by the presence in peptide ligands of A, L, I, V, M, S, or, T as a primary anchor at position 2, and a positively charged residue, R or K, at the C-terminal position of the epitope, *e.g.*, in position 9 of 9-mers (*see, e.g.*, Sidney *et al.*, *Hum. Immunol.* 45:79, 1996). Exemplary members of the corresponding family of HLA molecules (the HLA-A3 supertype) that bind the A3 supermotif include at least: A*0301, A*1101, A*3101, A*3301, and A*6801. Other allele-specific HLA molecules predicted to be members of the A3 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions of amino acids at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A3 supermotif are set forth on the attached Table IX.

IV.D.4. HLA-A24 supermotif

The HLA-A24 supermotif is characterized by the presence in peptide ligands of an aromatic (F, W, or Y) or hydrophobic aliphatic (L, I, V, M, or T) residue as a primary anchor in position 2, and Y, F, W, L, I, or M as primary anchor at the C-terminal position of the epitope (see, e.g., Sette and Sidney, *Immunogenetics*, in press, 1999). The corresponding family of HLA molecules that bind to the A24 supermotif (*i.e.*, the A24 supertype) includes at least: A*2402, A*3001, and A*2301. Other allele-specific HLA molecules predicted to be members of the A24 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the A24 supermotif are set forth on the attached Table X.

IV.D.5. HLA-B7 supermotif

The HLA-B7 supermotif is characterized by peptides bearing proline in position 2 as a primary anchor, and a hydrophobic or aliphatic amino acid (L, I, V, M, A, F, W, or Y) as the primary anchor at the C-terminal position of the epitope. The corresponding family of HLA molecules that bind the B7 supermotif (*i.e.*, the HLA-B7 supertype) is comprised of at least twenty six HLA-B proteins comprising at least: B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, and B*7801 (see, e.g., Sidney, *et al.*, *J. Immunol.* 154:247, 1995; Barber, *et al.*, *Curr. Biol.* 5:179, 1995; Hill, *et al.*, *Nature* 360:434, 1992; Rammensee, *et al.*, *Immunogenetics* 41:178, 1995 for reviews of relevant data). Other allele-specific HLA molecules predicted to be members of the B7 supertype are shown in Table VI. As explained in detail below, peptide binding to each of the individual allele-specific HLA proteins can be modulated by substitutions at the primary and/or secondary anchor positions of the peptide, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B7 supermotif are set forth on the attached Table XI.

IV.D.6. HLA-B27 supermotif

The HLA-B27 supermotif is characterized by the presence in peptide ligands of a positively charged (R, H, or K) residue as a primary anchor at position 2, and a hydrophobic (F, Y, L, W, M, I, A, or V) residue as a primary anchor at the C-terminal

5 position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999).

Exemplary members of the corresponding family of HLA molecules that bind to the B27 supermotif (*i.e.*, the B27 supertype) include at least B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, and B*7301. Other allele-specific HLA molecules predicted to be members of the B27 supertype are shown
10 in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Representative peptide epitopes that comprise the B27 supermotif are set forth on the attached Table XII.

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IV.D.7. HLA-B44 supermotif

The HLA-B44 supermotif is characterized by the presence in peptide ligands of negatively charged (D or E) residues as a primary anchor in position 2, and hydrophobic residues (F, W, Y, L, I, M, V, or A) as a primary anchor at the C-terminal position of the

20 epitope (*see, e.g.*, Sidney et al., *Immunol. Today* 17:261, 1996). Exemplary members of the corresponding family of HLA molecules that bind to the B44 supermotif (*i.e.*, the B44 supertype) include at least: B*1801, B*1802, B*3701, B*4001, B*4002, B*4006, B*4402, B*4403, and B*4404. Peptide binding to each of the allele-specific HLA
25 molecules can be modulated by substitutions at primary and/or secondary anchor positions; preferably choosing respective residues specified for the supermotif.

IV.D.8. HLA-B58 supermotif

The HLA-B58 supermotif is characterized by the presence in peptide ligands of a small aliphatic residue (A, S, or T) as a primary anchor residue at position 2, and an

30 aromatic or hydrophobic residue (F, W, Y, L, I, V, M, or A) as a primary anchor residue at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999 for reviews of relevant data). Exemplary members of the corresponding family of HLA molecules that bind to the B58 supermotif (*i.e.*, the B58 supertype) include at least: B*1516, B*1517, B*5701, B*5702, and B*5801. Other allele-specific

HLA molecules predicted to be members of the B58 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- 5 Representative peptide epitopes that comprise the B58 supermotif are set forth on the attached Table XIII.

IV.D.9. HLA-B62 supermotif

The HLA-B62 supermotif is characterized by the presence in peptide ligands of 10 the polar aliphatic residue Q or a hydrophobic aliphatic residue (L, V, M, I, or P) as a primary anchor in position 2, and a hydrophobic residue (F, W, Y, M, I, V, L, or A) as a primary anchor at the C-terminal position of the epitope (*see, e.g.*, Sidney and Sette, *Immunogenetics*, in press, 1999). Exemplary members of the corresponding family of HLA molecules that bind to the B62 supermotif (*i.e.*, the B62 supertype) include at least: 15 B*1501, B*1502, B*1513, and B5201. Other allele-specific HLA molecules predicted to be members of the B62 supertype are shown in Table VI. Peptide binding to each of the allele-specific HLA molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

- 20 Representative peptide epitopes that comprise the B62 supermotif are set forth on the attached Table XIV.

IV.D.10. HLA-A1 motif

The HLA-A1 motif is characterized by the presence in peptide ligands of T, S, or 25 M as a primary anchor residue at position 2 and the presence of Y as a primary anchor residue at the C-terminal position of the epitope. An alternative allele-specific A1 motif is characterized by a primary anchor residue at position 3 rather than position 2. This motif is characterized by the presence of D, E, A, or S as a primary anchor residue in position 3, and a Y as a primary anchor residue at the C-terminal position of the epitope 30 (*see, e.g.*, DiBrino *et al.*, J. Immunol., 152:620, 1994; Kondo *et al.*, *Immunogenetics* 45:249, 1997; and Kubo *et al.*, J. Immunol. 152:3913, 1994 for reviews of relevant data). Peptide binding to HLA-A1 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise either A1 motif are set forth on the attached Table XV. Those epitopes comprising T, S, or M at position 2 and Y at the C-terminal position are also included in the listing of HLA-A1 supermotif-bearing peptide epitopes listed in Table VII, as these residues are a subset of the A1 supermotif primary anchors.

IV.D.11. HLA-A*0201 motif

An HLA-A2*0201 motif was determined to be characterized by the presence in peptide ligands of L or M as a primary anchor residue in position 2, and L or V as a primary anchor residue at the C-terminal position of a 9-residue peptide (see, e.g., Falk *et al.*, *Nature* 351:290-296, 1991) and was further found to comprise an I at position 2 and I or A at the C-terminal position of a nine amino acid peptide (see, e.g., Hunt *et al.*, *Science* 255:1261-1263, March 6, 1992; Parker *et al.*, *J. Immunol.* 149:3580-3587, 1992). The A*0201 allele-specific motif has also been defined by the present inventors to additionally comprise V, A, T, or Q as a primary anchor residue at position 2, and M or T as a primary anchor residue at the C-terminal position of the epitope (see, e.g., Kast *et al.*, *J. Immunol.* 152:3904-3912, 1994). Thus, the HLA-A*0201 motif comprises peptide ligands with L, I, V, M, A, T, or Q as primary anchor residues at position 2 and L, I, V, M, A, or T as a primary anchor residue at the C-terminal position of the epitope. The preferred and tolerated residues that characterize the primary anchor positions of the HLA-A*0201 motif are identical to the residues describing the A2 supermotif. (For reviews of relevant data, see, e.g., del Guercio *et al.*, *J. Immunol.* 154:685-693, 1995; Ruppert *et al.*, *Cell* 74:929-937, 1993; Sidney *et al.*, *Immunol. Today* 17:261-266, 1996; Sette and Sidney, *Curr. Opin. in Immunol.* 10:478-482, 1998). Secondary anchor residues that characterize the A*0201 motif have additionally been defined (see, e.g., Ruppert *et al.*, *Cell* 74:929-937, 1993). These are shown in Table II. Peptide binding to HLA-A*0201 molecules can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise an A*0201 motif are set forth on the attached Table VIII. The A*0201 motifs comprising the primary anchor residues V, A, T, or Q at position 2 and L, I, V, A, or T at the C-terminal position are those most particularly relevant to the invention claimed herein.

IV.D.12. HLA-A3 motif

The HLA-A3 motif is characterized by the presence in peptide ligands of L, M, V, I, S, A, T, F, C, G, or D as a primary anchor residue at position 2, and the presence of K, sY, R, H, F, or A as a primary anchor residue at the C-terminal position of the epitope
 5 (see, e.g., DiBrino *et al.*, *Proc. Natl. Acad. Sci USA* 90:1508, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A3 motif are set forth on the
 10 attached Table XVI. Those peptide epitopes that also comprise the A3 supermotif are also listed in Table IX. The A3 supermotif primary anchor residues comprise a subset of the A3- and A11-allele specific motif primary anchor residues.

IV.D.13. HLA-A11 motif

The HLA-A11 motif is characterized by the presence in peptide ligands of V, T, M, L, I, S, A, G, N, C, D, or F as a primary anchor residue in position 2, and K, R, Y, or H as a primary anchor residue at the C-terminal position of the epitope (see, e.g., Zhang *et al.*, *Proc. Natl. Acad. Sci USA* 90:2217-2221, 1993; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A11 can be modulated by substitutions
 20 at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A11 motif are set forth on the attached Table XVII; peptide epitopes comprising the A3 allele-specific motif are also present in this Table because of the extensive overlap between the A3 and A11 motif
 25 primary anchor specificities. Further, those peptide epitopes that comprise the A3 supermotif are also listed in Table IX.

IV.D.14. HLA-A24 motif

The HLA-A24 motif is characterized by the presence in peptide ligands of Y, F, W, or M as a primary anchor residue in position 2, and F, L, I, or W as a primary anchor residue at the C-terminal position of the epitope (see, e.g., Kondo *et al.*, *J. Immunol.* 155:4307-4312, 1995; and Kubo *et al.*, *J. Immunol.* 152:3913-3924, 1994). Peptide binding to HLA-A24 molecules can be modulated by substitutions at primary and/or

secondary anchor positions; preferably choosing respective residues specified for the motif.

Representative peptide epitopes that comprise the A24 motif are set forth on the attached Table XVIII. These epitopes are also listed in Table X, which sets forth HLA-
5 A24-supermotif-bearing peptide epitopes, as the primary anchor residues characterizing the A24 allele-specific motif comprise a subset of the A24 supermotif primary anchor residues.

Motifs Indicative of Class II HLA Inducing Peptide Epitopes

10 The primary and secondary anchor residues of the HLA class II peptide epitope supermotifs and motifs delineated below are summarized in Table III.

IV.D.15. HLA DR-1-4-7 supermotif

Motifs have also been identified for peptides that bind to three common HLA
15 class II allele-specific HLA molecules: HLA DRB1*0401, DRB1*0101, and DRB1*0701
(see, e.g., the review by Southwood *et al. J. Immunology* 160:3363-3373,1998). Collectively, the common residues from these motifs delineate the HLA DR-1-4-7
supermotif. Peptides that bind to these DR molecules carry a supermotif characterized by
20 a large aromatic or hydrophobic residue (Y, F, W, L, I, V, or M) as a primary anchor residue in position 1, and a small, non-charged residue (S, T, C, A, P, V, I, L, or M) as a primary anchor residue in position 6 of a 9-mer core region. Allele-specific secondary effects and secondary anchors for each of these HLA types have also been identified
(Southwood *et al., supra*). These are set forth in Table III. Peptide binding to HLA-
DRB1*0401, DRB1*0101, and/or DRB1*0701 can be modulated by substitutions at
25 primary and/or secondary anchor positions, preferably choosing respective residues specified for the supermotif.

Potential epitope 9-mer core regions comprising the DR-1-4-7 supermotif, wherein position 1 of the supermotif is at position 1 of the nine-residue core, are set forth in Table XIX. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a c nine residue core, are also shown in the Table, along with cross-reactive binding data for the exemplary 15-residue supermotif-bearing peptides.

IV.D.16. HLA DR3 motifs

Two alternative motifs (*i.e.*, submotifs) characterize peptide epitopes that bind to HLA-DR3 molecules (*see, e.g.*, Geluk *et al.*, *J. Immunol.* 152:5742, 1994). In the first motif (submotif DR3a) a large, hydrophobic residue (L, I, V, M, F, or Y) is present in anchor position 1 of a 9-mer core, and D is present as an anchor at position 4, towards the carboxyl terminus of the epitope. As in other class II motifs, core position 1 may or may not occupy the peptide N-terminal position.

The alternative DR3 submotif provides for lack of the large, hydrophobic residue at anchor position 1, and/or lack of the negatively charged or amide-like anchor residue at position 4, by the presence of a positive charge at position 6 towards the carboxyl terminus of the epitope. Thus, for the alternative allele-specific DR3 motif (submotif DR3b): L, I, V, M, F, Y, A, or Y is present at anchor position 1; D, N, Q, E, S, or T is present at anchor position 4; and K, R, or H is present at anchor position 6. Peptide binding to HLA-DR3 can be modulated by substitutions at primary and/or secondary anchor positions, preferably choosing respective residues specified for the motif.

Potential peptide epitope 9-mer core regions corresponding to a nine residue sequence comprising the DR3a submotif (wherein position 1 of the motif is at position 1 of the nine residue core) are set forth in Table XXa. Respective exemplary peptide epitopes of 15 amino acid residues in length, each of which comprise a nine residue core, are also shown in Table XXa along with binding data of the exemplary DR3 submotif a-bearing peptides.

Potential peptide epitope 9-mer core regions comprising the DR3b submotif and respective exemplary 15-mer peptides comprising the DR3 submotif-b epitope are set forth in Table XXb. Binding data of exemplary DR3 submotif b-bearing peptides is also shown.

Each of the HLA class I or class II peptide epitopes set out in the Tables herein are deemed singly to be an inventive aspect of this application. Further, it is also an inventive aspect of this application that each peptide epitope may be used in combination with any other peptide epitope.

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IV.E. Enhancing Population Coverage of the Vaccine

Vaccines that have broad population coverage are preferred because they are more commercially viable and generally applicable to the most people. Broad population coverage can be obtained using the peptides of the invention (and nucleic acid

compositions that encode such peptides) through selecting peptide epitopes that bind to HLA alleles which, when considered in total, are present in most of the population. Table XXI lists the overall frequencies of the HLA class I supertypes in various ethnicities (Table XXIa) and the combined population coverage achieved by the A2-, A3-, and B7- supertypes (Table XXIb). The A2-, A3-, and B7 supertypes are each present on the average of over 40% in each of these five major ethnic groups. Coverage in excess of 80% is achieved with a combination of these supermotifs. These results suggest that effective and non-ethnically biased population coverage is achieved upon use of a limited number of cross-reactive peptides. Although the population coverage reached with these three main peptide specificities is high, coverage can be expanded to reach 95% population coverage and above, and more easily achieve truly multispecific responses upon use of additional supermotif or allele-specific motif bearing peptides.

The B44-, A1-, and A24-supertypes are each present, on average, in a range from 25% to 40% in these major ethnic populations (Table XXIa). While less prevalent overall, the B27-, B58-, and B62 supertypes are each present with a frequency >25% in at least one major ethnic group (Table XXIa). Table XXIb summarizes the estimated prevalence of combinations of HLA supertypes that have been identified in five major ethnic groups. The incremental coverage obtained by the inclusion of A1-, A24-, and B44-supertypes to the A2, A3, and B7 coverage and coverage obtained with all of the supertypes described herein, is shown.

The data presented herein, together with the previous definition of the A2-, A3-, and B7-supertypes, indicates that all antigens, with the possible exception of A29, B8, and B46, can be classified into a total of nine HLA supertypes. By including epitopes from the six most frequent supertypes, an average population coverage of 99% is obtained for five major ethnic groups.

IV.F. Immune Response-Stimulating Peptide Analogs

In general, CTL and HTL responses are not directed against all possible epitopes. Rather, they are restricted to a few “immunodominant” determinants (Zinkernagel, *et al.*, 30 *Adv. Immunol.* 27:5159, 1979; Bennink, *et al.*, *J. Exp. Med.* 168:19351939, 1988; Rawle, *et al.*, *J. Immunol.* 146:3977-3984, 1991). It has been recognized that immunodominance (Benacerraf, *et al.*, *Science* 175:273-279, 1972) could be explained by either the ability of a given epitope to selectively bind a particular HLA protein (determinant selection theory) (Vitiello, *et al.*, *J. Immunol.* 131:1635, 1983); Rosenthal, *et al.*, *Nature* 267:156-

158, 1977), or to be selectively recognized by the existing TCR (T cell receptor) specificities (repertoire theory) (Klein, J., IMMUNOLOGY, THE SCIENCE OF SELF/NONSELF DISCRIMINATION, John Wiley & Sons, New York, pp. 270-310, 1982). It has been demonstrated that additional factors, mostly linked to processing events, can also play a 5 key role in dictating, beyond strict immunogenicity, which of the many potential determinants will be presented as immunodominant (Sercarz, *et al.*, *Annu. Rev. Immunol.* 11:729-766, 1993).

Because tissue specific and developmental TAAs are expressed on normal tissue at least at some point in time or location within the body, it may be expected that T cells 10 to them, particularly dominant epitopes, are eliminated during immunological surveillance and that tolerance is induced. However, CTL responses to tumor epitopes in both normal donors and cancer patient has been detected, which may indicate that tolerance is incomplete (see, e.g., Kawashima *et al.*, *Hum. Immunol.* 59:1, 1998; Tsang, *J. Natl. Cancer Inst.* 87:82-90, 1995; Rongcun *et al.*, *J. Immunol.* 163:1037, 1999). Thus, 15 immune tolerance does not completely eliminate or inactivate CTL precursors capable of recognizing high affinity HLA class I binding peptides.

An additional strategy to overcome tolerance is to use analog peptides. Without intending to be bound by theory, it is believed that because T cells to dominant epitopes may have been clonally deleted, selecting subdominant epitopes may allow existing T 20 cells to be recruited, which will then lead to a therapeutic or prophylactic response. However, the binding of HLA molecules to subdominant epitopes is often less vigorous than to dominant ones. Accordingly, there is a need to be able to modulate the binding affinity of particular immunogenic epitopes for one or more HLA molecules, and thereby 25 to modulate the immune response elicited by the peptide, for example to prepare analog peptides which elicit a more vigorous response.

Although peptides with suitable cross-reactivity among all alleles of a superfamily are identified by the screening procedures described above, cross-reactivity is not always as complete as possible, and in certain cases procedures to increase cross-reactivity of peptides can be useful; moreover, such procedures can also be used to modify other 30 properties of the peptides such as binding affinity or peptide stability. Having established the general rules that govern cross-reactivity of peptides for HLA alleles within a given motif or supermotif, modification (*i.e.*, analoging) of the structure of peptides of particular interest in order to achieve broader (or otherwise modified) HLA binding capacity can be performed. More specifically, peptides which exhibit the broadest cross-

reactivity patterns, can be produced in accordance with the teachings herein. The present concepts related to analog generation are set forth in greater detail in co-pending U.S.S.N. 09/226,775 filed 1/6/99.

In brief, the strategy employed utilizes the motifs or supermotifs which correlate
5 with binding to certain HLA molecules. The motifs or supermotifs are defined by having primary anchors, and in many cases secondary anchors. Analog peptides can be created by substituting amino acid residues at primary anchor, secondary anchor, or at primary and secondary anchor positions. Generally, analogs are made for peptides that already bear a motif or supermotif. Preferred secondary anchor residues of supermotifs and
10 motifs that have been defined for HLA class I and class II binding peptides are shown in Tables II and III, respectively.

For a number of the motifs or supermotifs in accordance with the invention, residues are defined which are deleterious to binding to allele-specific HLA molecules or members of HLA supertypes that bind the respective motif or supermotif (Tables II and III). Accordingly, removal of such residues that are detrimental to binding can be performed in accordance with the present invention. For example, in the case of the A3 supertype, when all peptides that have such deleterious residues are removed from the population of peptides used in the analysis, the incidence of cross-reactivity increased from 22% to 37% (see, e.g., Sidney, J. et al., *Hu. Immunol.* 45:79, 1996). Thus, one strategy to improve the cross-reactivity of peptides within a given supermotif is simply to delete one or more of the deleterious residues present within a peptide and substitute a small "neutral" residue such as Ala (that may not influence T cell recognition of the peptide). An enhanced likelihood of cross-reactivity is expected if, together with elimination of detrimental residues within a peptide, "preferred" residues associated with
20 high affinity binding to an allele-specific HLA molecule or to multiple HLA molecules within a superfamily are inserted.
25

To ensure that an analog peptide, when used as a vaccine, actually elicits a CTL response to the native epitope *in vivo* (or, in the case of class II epitopes, elicits helper T cells that cross-react with the wild type peptides), the analog peptide may be used to immunize T cells *in vitro* from individuals of the appropriate HLA allele. Thereafter, the immunized cells' capacity to induce lysis of wild type peptide sensitized target cells is evaluated. It will be desirable to use as antigen presenting cells, cells that have been either infected, or transfected with the appropriate genes, or, in the case of class II

epitopes only, cells that have been pulsed with whole protein antigens, to establish whether endogenously produced antigen is also recognized by the relevant T cells.

Another embodiment of the invention is to create analogs of weak binding peptides, to thereby ensure adequate numbers of cross-reactive cellular binders. Class I binding peptides exhibiting binding affinities of 500-5000 nM, and carrying an acceptable but suboptimal primary anchor residue at one or both positions can be “fixed” by substituting preferred anchor residues in accordance with the respective supertype. The analog peptides can then be tested for crossbinding activity.

Another embodiment for generating effective peptide analogs involves the substitution of residues that have an adverse impact on peptide stability or solubility in, e.g., a liquid environment. This substitution may occur at any position of the peptide epitope. For example, a cysteine can be substituted out in favor of α -amino butyric acid (“B” in the single letter abbreviations for peptide sequences listed herein). Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substituting α -amino butyric acid for cysteine not only alleviates this problem, but actually improves binding and crossbinding capability in certain instances (see, e.g., the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

Representative analog peptides are set forth in Table XXII. The Table indicates the length and sequence of the analog peptide as well as the motif or supermotif, if appropriate. The information in the “Fixed Nomenclature” column indicates the residues substituted at the indicated position numbers for the respective analog.

IV.G. Computer Screening of Protein Sequences from Disease-Related Antigens for Supermotif- or Motif-Bearing Peptides

In order to identify supermotif- or motif-bearing epitopes in a target antigen, a native protein sequence, e.g., a tumor-associated antigen, or sequences from an infectious organism, or a donor tissue for transplantation, is screened using a means for computing, such as an intellectual calculation or a computer, to determine the presence of a supermotif or motif within the sequence. The information obtained from the analysis of native peptide can be used directly to evaluate the status of the native peptide or may be utilized subsequently to generate the peptide epitope.

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Computer programs that allow the rapid screening of protein sequences for the occurrence of the subject supermotifs or motifs are encompassed by the present invention; as are programs that permit the generation of analog peptides. These programs are implemented to analyze any identified amino acid sequence or operate on an unknown sequence and simultaneously determine the sequence and identify motif-bearing epitopes thereof; analogs can be simultaneously determined as well. Generally, the identified sequences will be from a pathogenic organism or a tumor-associated peptide. For example, the target TAA molecules include, without limitation, CEA, MAGE, p53 and HER2/neu.

It is important that the selection criteria utilized for prediction of peptide binding are as accurate as possible, to correlate most efficiently with actual binding. Prediction of peptides that bind, for example, to HLA-A*0201, on the basis of the presence of the appropriate primary anchors, is positive at about a 30% rate (*see, e.g.*, Ruppert, J. *et al.* *Cell* 74:929, 1993). However, by extensively analyzing peptide-HLA binding data disclosed herein, data in related patent applications, and data in the art, the present inventors have developed a number of allele-specific polynomial algorithms that dramatically increase the predictive value over identification on the basis of the presence of primary anchor residues alone. These algorithms take into account not only the presence or absence of primary anchors, but also consider the positive or deleterious presence of secondary anchor residues (to account for the impact of different amino acids at different positions). The algorithms are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient that represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. An important assumption of this method is that the effects at each position are essentially independent of each other. This assumption is justified by studies that demonstrated that peptides are bound to HLA molecules and recognized by T cells in essentially an extended conformation. Derivation of specific algorithm coefficients has been described, for example, in Gulukota, K. *et al.*, *J. Mol. Biol.* 267:1258, 1997.

Additional methods to identify preferred peptide sequences, which also make use of specific motifs, include the use of neural networks and molecular modeling programs

(see, e.g., Milik *et al.*, *Nature Biotechnology* 16:753, 1998; Altuvia *et al.*, *Hum. Immunol.* 58:1, 1997; Altuvia *et al.*, *J. Mol. Biol.* 249:244, 1995; Buus, S. *Curr. Opin. Immunol.* 11:209-213, 1999; Brusic, V. *et al.*, *Bioinformatics* 14:121-130, 1998; Parker *et al.*, *J. Immunol.* 152:163, 1993; Meister *et al.*, *Vaccine* 13:581, 1995; Hammer *et al.*, *J. Exp. Med.* 180:2353, 1994; Sturniolo *et al.*, *Nature Biotechnol.* 17:555 1999).

For example, it has been shown that in sets of A*0201 motif-bearing peptides containing at least one preferred secondary anchor residue while avoiding the presence of any deleterious secondary anchor residues, 69% of the peptides will bind A*0201 with an IC₅₀ less than 500 nM (Ruppert, J. *et al.* *Cell* 74:929, 1993). These algorithms are also flexible in that cut-off scores may be adjusted to select sets of peptides with greater or lower predicted binding properties, as desired.

In utilizing computer screening to identify peptide epitopes, a protein sequence or translated sequence may be analyzed using software developed to search for motifs, for example the "FINDPATTERNS" program (Devereux, *et al.* *Nucl. Acids Res.* 12:387-395, 1984) or MotifSearch 1.4 software program (D. Brown, San Diego, CA) to identify potential peptide sequences containing appropriate HLA binding motifs. The identified peptides can be scored using customized polynomial algorithms to predict their capacity to bind specific HLA class I or class II alleles. As appreciated by one of ordinary skill in the art, a large array of computer programming software and hardware options are available in the relevant art which can be employed to implement the motifs of the invention in order to evaluate (e.g., without limitation, to identify epitopes, identify epitope concentration per peptide length, or to generate analogs) known or unknown peptide sequences.

In accordance with the procedures described above, MAGE2/3 peptide epitopes and analogs thereof that are able to bind HLA supertype groups or allele-specific HLA molecules have been identified (Tables VII-XX; Table XXII).

IV.H. Preparation of Peptide Epitopes

Peptides in accordance with the invention can be prepared synthetically, by recombinant DNA technology or chemical synthesis, or from natural sources such as native tumors or pathogenic organisms. Peptide epitopes may be synthesized individually or as polyepitopic peptides. Although the peptide will preferably be substantially free of other naturally occurring host cell proteins and fragments thereof, in some embodiments the peptides may be synthetically conjugated to native fragments or particles.

The peptides in accordance with the invention can be a variety of lengths, and either in their neutral (uncharged) forms or in forms which are salts. The peptides in accordance with the invention are either free of modifications such as glycosylation, side chain oxidation, or phosphorylation; or they contain these modifications, subject to the condition that modifications do not destroy the biological activity of the peptides as described herein.

Desirably, the peptide epitope will be as small as possible while still maintaining substantially all of the immunologic activity of the native protein. When possible, it may be desirable to optimize HLA class I binding peptide epitopes of the invention to a length of about 8 to about 13 amino acid residues, preferably 9 to 10. HLA class II binding peptide epitopes may be optimized to a length of about 6 to about 30 amino acids in length, preferably to between about 13 and about 20 residues. Preferably, the peptide epitopes are commensurate in size with endogenously processed pathogen-derived peptides or tumor cell peptides that are bound to the relevant HLA molecules.

The identification and preparation of peptides of other lengths can also be carried out using the techniques described herein. Moreover, it is preferred to identify native peptide regions that contain a high concentration of class I and/or class II epitopes. Such a sequence is generally selected on the basis that it contains the greatest number of epitopes per amino acid length. It is to be appreciated that epitopes can be present in a frame-shifted manner, *e.g.* a 10 amino acid long peptide could contain two 9 amino acid long epitopes and one 10 amino acid long epitope; upon intracellular processing, each epitope can be exposed and bound by an HLA molecule upon administration of such a peptide. This larger, preferably multi-epitopic, peptide can be generated synthetically, recombinantly, or via cleavage from the native source.

The peptides of the invention can be prepared in a wide variety of ways. For the preferred relatively short size, the peptides can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automatic synthesizers are commercially available and can be used in accordance with known protocols. (*See, for example, Stewart & Young, SOLID PHASE PEPTIDE SYNTHESIS, 2D. ED., Pierce Chemical Co., 1984.*) Further, individual peptide epitopes can be joined using chemical ligation to produce larger peptides that are still within the bounds of the invention.

Alternatively, recombinant DNA technology can be employed wherein a nucleotide sequence which encodes an immunogenic peptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell and cultivated

under conditions suitable for expression. These procedures are generally known in the art, as described generally in Sambrook *et al.*, MOLECULAR CLONING, A LABORATORY MANUAL, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989). Thus, recombinant polypeptides which comprise one or more peptide sequences of the invention can be used to present the appropriate T cell epitope.

The nucleotide coding sequence for peptide epitopes of the preferred lengths contemplated herein can be synthesized by chemical techniques, for example, the phosphotriester method of Matteucci, *et al.*, *J. Am. Chem. Soc.* 103:3185 (1981). Peptide analogs can be made simply by substituting the appropriate and desired nucleic acid base(s) for those that encode the native peptide sequence; exemplary nucleic acid substitutions are those that encode an amino acid defined by the motifs/supermotifs herein. The coding sequence can then be provided with appropriate linkers and ligated into expression vectors commonly available in the art, and the vectors used to transform suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are now available. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions and usually a replication system to provide an expression vector for expression in the desired cellular host. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect or mammalian cell hosts may also be used, employing suitable vectors and control sequences.

IV.I. Assays to Detect T-Cell Responses

Once HLA binding peptides are identified, they can be tested for the ability to elicit a T-cell response. The preparation and evaluation of motif-bearing peptides are described in PCT publications WO 94/20127 and WO 94/03205. Briefly, peptides comprising epitopes from a particular antigen are synthesized and tested for their ability to bind to the appropriate HLA proteins. These assays may involve evaluating the binding of a peptide of the invention to purified HLA class I molecules in relation to the binding of a radioiodinated reference peptide. Alternatively, cells expressing empty class I molecules (*i.e.* lacking peptide therein) may be evaluated for peptide binding by immunofluorescent staining and flow microfluorimetry. Other assays that may be used to evaluate peptide binding include peptide-dependent class I assembly assays and/or the

inhibition of CTL recognition by peptide competition. Those peptides that bind to the class I molecule, typically with an affinity of 500 nM or less, are further evaluated for their ability to serve as targets for CTLs derived from infected or immunized individuals, as well as for their capacity to induce primary *in vitro* or *in vivo* CTL responses that can give rise to CTL populations capable of reacting with selected target cells associated with a disease. Corresponding assays are used for evaluation of HLA class II binding peptides. HLA class II motif-bearing peptides that are shown to bind, typically at an affinity of 1000 nM or less, are further evaluated for the ability to stimulate HTL responses.

Conventional assays utilized to detect T cell responses include proliferation assays, lymphokine secretion assays, direct cytotoxicity assays, and limiting dilution assays. For example, antigen-presenting cells that have been incubated with a peptide can be assayed for the ability to induce CTL responses in responder cell populations.

Antigen-presenting cells can be normal cells such as peripheral blood mononuclear cells or dendritic cells. Alternatively, mutant non-human mammalian cell lines that are deficient in their ability to load class I molecules with internally processed peptides and that have been transfected with the appropriate human class I gene, may be used to test for the capacity of the peptide to induce *in vitro* primary CTL responses.

Peripheral blood mononuclear cells (PBMCs) may be used as the responder cell source of CTL precursors. The appropriate antigen-presenting cells are incubated with peptide, after which the peptide-loaded antigen-presenting cells are then incubated with the responder cell population under optimized culture conditions. Positive CTL activation can be determined by assaying the culture for the presence of CTLs that kill radio-labeled target cells, both specific peptide-pulsed targets as well as target cells expressing endogenously processed forms of the antigen from which the peptide sequence was derived.

More recently, a method has been devised which allows direct quantification of antigen-specific T cells by staining with Fluorescein-labelled HLA tetrameric complexes (Altman, J. D. *et al.*, *Proc. Natl. Acad. Sci. USA* 90:10330, 1993; Altman, J. D. *et al.*, *Science* 274:94, 1996). Other relatively recent technical developments include staining for intracellular lymphokines, and interferon- γ release assays or ELISPOT assays. Tetramer staining, intracellular lymphokine staining and ELISPOT assays all appear to be at least 10-fold more sensitive than more conventional assays (Lalvani, A. *et al.*, *J. Exp.*

Med. 186:859, 1997; Dunbar, P. R. *et al.*, *Curr. Biol.* 8:413, 1998; Murali-Krishna, K. *et al.*, *Immunity* 8:177, 1998).

HTL activation may also be assessed using such techniques known to those in the art such as T cell proliferation and secretion of lymphokines, e.g. IL-2 (*see, e.g.*

5 Alexander *et al.*, *Immunity* 1:751-761, 1994).

Alternatively, immunization of HLA transgenic mice can be used to determine immunogenicity of peptide epitopes. Several transgenic mouse models including mice with human A2.1, A11 (which can additionally be used to analyze HLA-A3 epitopes), and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and 10 A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have also been developed. Additional transgenic mouse models with other HLA alleles may be generated as necessary. Mice may be immunized with peptides emulsified in Incomplete Freund's Adjuvant and the resulting T cells tested for their capacity to recognize peptide-pulsed target cells and target cells transfected with appropriate genes. CTL responses 15 may be analyzed using cytotoxicity assays described above. Similarly, HTL responses may be analyzed using such assays as T cell proliferation or secretion of lymphokines.

Exemplary immunogenic peptide epitopes are set out in Table XXIII.

IV.J. Use of Peptide Epitopes as Diagnostic Agents and for Evaluating Immune 20 Responses

HLA class I and class II binding peptides as described herein can be used, in one embodiment of the invention, as reagents to evaluate an immune response. The immune response to be evaluated may be induced by using as an immunogen any agent that may result in the production of antigen-specific CTLs or HTLs that recognize and bind to the 25 peptide epitope(s) to be employed as the reagent. The peptide reagent need not be used as the immunogen. Assay systems that may be used for such an analysis include relatively recent technical developments such as tetramers, staining for intracellular lymphokines and interferon release assays, or ELISPOT assays.

For example, a peptide of the invention may be used in a tetramer staining assay 30 to assess peripheral blood mononuclear cells for the presence of antigen-specific CTLs following exposure to a tumor cell antigen or an immunogen. The HLA-tetrameric complex is used to directly visualize antigen-specific CTLs (*see, e.g.*, Ogg *et al.*, *Science* 279:2103-2106, 1998; and Altman *et al.*, *Science* 174:94-96, 1996) and determine the frequency of the antigen-specific CTL population in a sample of peripheral blood

mononuclear cells. A tetramer reagent using a peptide of the invention may be generated as follows: A peptide that binds to an HLA molecule is refolded in the presence of the corresponding HLA heavy chain and β_2 -microglobulin to generate a trimolecular complex. The complex is biotinylated at the carboxyl terminal end of the heavy chain at 5 a site that was previously engineered into the protein. Tetramer formation is then induced by the addition of streptavidin. By means of fluorescently labeled streptavidin, the tetramer can be used to stain antigen-specific cells. The cells may then be identified, for example, by flow cytometry. Such an analysis may be used for diagnostic or prognostic purposes.

10 Peptides of the invention may also be used as reagents to evaluate immune recall responses (see, e.g., Bertoni *et al.*, *J. Clin. Invest.* 100:503-513, 1997 and Penna *et al.*, *J. Exp. Med.* 174:1565-1570, 1991). For example, patient PBMC samples from individuals with cancer may be analyzed for the presence of antigen-specific CTLs or HTLs using specific peptides. A blood sample containing mononuclear cells may be evaluated by cultivating the PBMCs and stimulating the cells with a peptide of the invention. After an appropriate cultivation period, the expanded cell population may be analyzed, for 15 example, for CTL or for HTL activity.

The peptides may also be used as reagents to evaluate the efficacy of a vaccine. PBMCs obtained from a patient vaccinated with an immunogen may be analyzed using, 20 for example, either of the methods described above. The patient is HLA typed, and peptide epitope reagents that recognize the allele-specific molecules present in that patient are selected for the analysis. The immunogenicity of the vaccine is indicated by the presence of epitope-specific CTLs and/or HTLs in the PBMC sample.

The peptides of the invention may also be used to make antibodies, using 25 techniques well known in the art (see, e.g. *CURRENT PROTOCOLS IN IMMUNOLOGY*, Wiley/Greene, NY; and *Antibodies A Laboratory Manual*, Harlow and Lane, Cold Spring Harbor Laboratory Press, 1989), which may be useful as reagents to diagnose or monitor cancer. Such antibodies include those that recognize a peptide in the context of an HLA molecule, *i.e.*, antibodies that bind to a peptide-MHC complex.

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IV.K. Vaccine Compositions

Vaccines that contain an immunogenically effective amount of one or more peptides as described herein are a further embodiment of the invention. Once

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appropriately immunogenic epitopes have been defined, they can be sorted and delivered by various means, herein referred to as "vaccine" compositions. Such vaccine compositions can include, for example, lipopeptides (e.g., Vitiello, A. *et al.*, *J. Clin. Invest.* 95:341, 1995), peptide compositions encapsulated in poly(DL-lactide-co-glycolide) ("PLG") microspheres (see, e.g., Eldridge, *et al.*, *Molec. Immunol.* 28:287-294, 1991; Alonso *et al.*, *Vaccine* 12:299-306, 1994; Jones *et al.*, *Vaccine* 13:675-681, 1995), peptide compositions contained in immune stimulating complexes (ISCOMS) (see, e.g., Takahashi *et al.*, *Nature* 344:873-875, 1990; Hu *et al.*, *Clin Exp Immunol.* 113:235-243, 1998), multiple antigen peptide systems (MAPs) (see e.g., Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.* 85:5409-5413, 1988; Tam, J.P., *J. Immunol. Methods* 196:17-32, 1996), viral delivery vectors (Perkus, M. E. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 379, 1996; Chakrabarti, S. *et al.*, *Nature* 320:535, 1986; Hu, S. L. *et al.*, *Nature* 320:537, 1986; Kieny, M.-P. *et al.*, *AIDS Bio/Technology* 4:790, 1986; Top, F. H. *et al.*, *J. Infect. Dis.* 124:148, 1971; Chanda, P. K. *et al.*, *Virology* 175:535, 1990), particles of viral or synthetic origin (e.g., Kofler, N. *et al.*, *J. Immunol. Methods*. 192:25, 1996; Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993; Falo, L. D., Jr. *et al.*, *Nature Med.* 7:649, 1995), adjuvants (Warren, H. S., Vogel, F. R., and Chedid, L. A. *Annu. Rev. Immunol.* 4:369, 1986; Gupta, R. K. *et al.*, *Vaccine* 11:293, 1993), liposomes (Reddy, R. *et al.*, *J. Immunol.* 148:1585, 1992; Rock, K. L., *Immunol. Today* 17:131, 1996), or, naked or particle absorbed cDNA (Ulmer, J. B. *et al.*, *Science* 259:1745, 1993; Robinson, H. L., Hunt, L. A., and Webster, R. G., *Vaccine* 11:957, 1993; Shiver, J. W. *et al.*, In: *Concepts in vaccine development*, Kaufmann, S. H. E., ed., p. 423, 1996; Cease, K. B., and Berzofsky, J. A., *Annu. Rev. Immunol.* 12:923, 1994 and Eldridge, J. H. *et al.*, *Sem. Hematol.* 30:16, 1993). Toxin-targeted delivery technologies, also known as receptor mediated targeting, such as those of Avant Immunotherapeutics, Inc. (Needham, Massachusetts) may also be used.

Furthermore, vaccines in accordance with the invention encompass compositions of one or more of the claimed peptide(s). The peptide(s) can be individually linked to its own carrier; alternatively, the peptide(s) can exist as a homopolymer or heteropolymer of active peptide units. Such a polymer has the advantage of increased immunological reaction and, where different peptide epitopes are used to make up the polymer, the additional ability to induce antibodies and/or CTLs that react with different antigenic determinants of the pathogenic organism or tumor-related peptide targeted for an immune

response. The composition may be a naturally occurring region of an antigen or may be prepared, *e.g.*, recombinantly or by chemical synthesis.

Furthermore, useful carriers that can be used with vaccines of the invention are well known in the art, and include, *e.g.*, thyroglobulin, albumins such as human serum 5 albumin, tetanus toxoid, polyamino acids such as poly L-lysine, poly L-glutamic acid, influenza, hepatitis B virus core protein, and the like. The vaccines can contain a physiologically tolerable (*i.e.*, acceptable) diluent such as water, or saline, preferably phosphate buffered saline. The vaccines also typically include an adjuvant. Adjuvants such as incomplete Freund's adjuvant, aluminum phosphate, aluminum hydroxide, or 10 alum are examples of materials well known in the art. Additionally, as disclosed herein, CTL responses can be primed by conjugating peptides of the invention to lipids, such as tripalmitoyl-S-glycercylcysteinylseryl-serine (P₃CSS).

As disclosed in greater detail herein, upon immunization with a peptide composition in accordance with the invention, via injection, aerosol, oral, transdermal, 15 transmucosal, intrapleural, intrathecal, or other suitable routes, the immune system of the host responds to the vaccine by producing large amounts of CTLs and/or HTLs specific for the desired antigen. Consequently, the host becomes at least partially immune to later infection, or at least partially resistant to developing an ongoing chronic infection, or derives at least some therapeutic benefit when the antigen was tumor-associated.

In some instances it may be desirable to combine the class I peptide vaccines of 20 the invention with vaccines which induce or facilitate neutralizing antibody responses to the target antigen of interest, particularly to viral envelope antigens. A preferred embodiment of such a composition comprises class I and class II epitopes in accordance with the invention. An alternative embodiment of such a composition comprises a class I 25 and/or class II epitope in accordance with the invention, along with a PADRE™ (Epimmune, San Diego, CA) molecule (described, for example, in U.S. Patent Number 5,736,142). Furthermore, any of these embodiments can be administered as a nucleic acid mediated modality.

For therapeutic or prophylactic immunization purposes, the peptides of the 30 invention can also be expressed by viral or bacterial vectors. Examples of expression vectors include attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of vaccinia virus, for example, as a vector to express nucleotide sequences that encode the peptides of the invention. Upon introduction into a host

bearing a tumor, the recombinant vaccinia virus expresses the immunogenic peptide, and thereby elicits a host CTL and/or HTL response. Vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4,722,848. Another vector is BCG (Bacille Calmette Guerin). BCG vectors are described in Stover *et al.*, *Nature* 351:456-460 (1991). A wide variety of other vectors useful for therapeutic administration or immunization of the peptides of the invention, e.g. adeno and adeno-associated virus vectors, retroviral vectors, *Salmonella typhi* vectors, detoxified anthrax toxin vectors, and the like, will be apparent to those skilled in the art from the description herein.

Antigenic peptides are used to elicit a CTL and/or HTL response *ex vivo*, as well. The resulting CTL or HTL cells, can be used to treat chronic infections, or tumors in patients that do not respond to other conventional forms of therapy, or will not respond to a therapeutic vaccine peptide or nucleic acid in accordance with the invention. *Ex vivo* CTL or HTL responses to a particular antigen (infectious or tumor-associated antigen) are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptide. After an appropriate incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cell (an infected cell or a tumor cell). Transfected dendritic cells may also be used as antigen presenting cells. Alternatively, dendritic cells are transfected, e.g., with a minigene construct in accordance with the invention, in order to elicit immune responses. Minigenes will be discussed in greater detail in a following section.

Vaccine compositions may also be administered *in vivo* in combination with dendritic cell mobilization whereby loading of dendritic cells occurs *in vivo*.

DNA or RNA encoding one or more of the peptides of the invention can also be administered to a patient. This approach is described, for instance, in Wolff *et. al.*, *Science* 247:1465 (1990) as well as U.S. Patent Nos. 5,580,859; 5,589,466; 5,804,566; 5,739,118; 5,736,524; 5,679,647; WO 98/04720; and in more detail below. Examples of DNA-based delivery technologies include “naked DNA”, facilitated (bupivacaine, polymers, peptide-mediated) delivery, cationic lipid complexes, and particle-mediated (“gene gun”) or pressure-mediated delivery (*see, e.g.*, U.S. Patent No. 5,922,687).

Preferably, the following principles are utilized when selecting an array of epitopes for inclusion in a polyepitopic composition for use in a vaccine, or for selecting

discrete epitopes to be included in a vaccine and/or to be encoded by nucleic acids such as a minigene. Exemplary epitopes that may be utilized in a vaccine to treat or prevent cancer are set out in Tables XXXVII and XXXVIII. It is preferred that each of the following principles are balanced in order to make the selection. The multiple epitopes to 5 be incorporated in a given vaccine composition may be, but need not be, contiguous in sequence in the native antigen from which the epitopes are derived.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar 10 rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*, Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15. The MAGE2/3 epitopes selected for inclusion are 15 preferably conserved between the two proteins.

2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC₅₀ of 500 nM or less, or for Class II an IC₅₀ of 1000 nM or less.

3.) Sufficient supermotif bearing-peptides, or a sufficient array of allele-specific motif-bearing peptides, are selected to give broad population coverage. For example, it is preferable to have at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art, can be employed to assess the breadth, or redundancy of, population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred 25 to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of particular relevance for infectious disease vaccines (but for cancer-related vaccines as well), are epitopes referred to as “nested epitopes.” Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A 30 peptide comprising “transcendent nested epitopes” is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, it is preferable to provide a sequence that has the greatest number of epitopes per provided sequence. Preferably, one avoids providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the

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carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, it is important to screen the sequence in order to insure that it does not have pathological or other deleterious biological properties.

5 . . . When creating a minigene, as disclosed in greater detail in the following section, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Furthermore, upon determination of the nucleic acid sequence to be provided as a minigene, the peptide
10 encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, e.g., by motif analysis, that only exists because two discrete peptide sequences are encoded directly next to each other. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that non-native epitope. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.
15

IV.K.1. Minigene Vaccines

20 A growing body of experimental evidence demonstrates that a number of different approaches are available which allow simultaneous delivery of multiple epitopes. Nucleic acids encoding the peptides of the invention are a particularly useful embodiment of the invention. Epitopes for inclusion in a minigene are preferably selected according to the guidelines set forth in the previous section. A preferred means of administering
25 nucleic acids encoding the peptides of the invention uses minigene constructs encoding a peptide comprising one or multiple epitopes of the invention. The use of multi-epitope minigenes is described below and in, e.g., co-pending application U.S.S.N. 09/311,784; Ishioka *et al.*, *J. Immunol.* 162:3915-3925, 1999; An, L. and Whitton, J. L., *J. Virol.* 71:2292, 1997; Thomson, S. A. *et al.*, *J. Immunol.* 157:822, 1996; Whitton, J. L. *et al.*, *J.*
30 *Virol.* 67:348, 1993; Hanke, R. *et al.*, *Vaccine* 16:426, 1998. For example, a multi-epitope DNA plasmid encoding supermotif- and/or motif-bearing MAGE2/3 epitopes derived from multiple regions of the MAGE2/3 proteins, the PADRE™ universal helper T cell epitope (or multiple HTL epitopes from MAGE2/3), and an endoplasmic reticulum-

translocating signal sequence can be engineered. A vaccine may also comprise epitopes, in addition to MAGE2/3 epitopes, that are derived from other TAAs.

The immunogenicity of a multi-epitopic minigene can be tested in transgenic mice to evaluate the magnitude of CTL induction responses against the epitopes tested.

- 5 Further, the immunogenicity of DNA-encoded epitopes *in vivo* can be correlated with the
in vitro responses of specific CTL lines against target cells transfected with the DNA
plasmid. Thus, these experiments can show that the minigene serves to both: 1.) generate
a CTL response and 2.) that the induced CTLs recognized cells expressing the encoded
epitopes.

10 For example, to create a DNA sequence encoding the selected epitopes (minigene)
for expression in human cells, the amino acid sequences of the epitopes may be reverse
translated. A human codon usage table can be used to guide the codon choice for each
amino acid. These epitope-encoding DNA sequences may be directly adjoined, so that
when translated, a continuous polypeptide sequence is created. To optimize expression
15 and/or immunogenicity, additional elements can be incorporated into the minigene
design. Examples of amino acid sequences that can be reverse translated and included in
the minigene sequence include: HLA class I epitopes, HLA class II epitopes, a
ubiquitination signal sequence, and/or an endoplasmic reticulum targeting signal. In
addition, HLA presentation of CTL and HTL epitopes may be improved by including
synthetic (*e.g.* poly-alanine) or naturally-occurring flanking sequences adjacent to the
20 CTL or HTL epitopes; these larger peptides comprising the epitope(s) are within the
scope of the invention.

The minigene sequence may be converted to DNA by assembling oligonucleotides that encode the plus and minus strands of the minigene. Overlapping oligonucleotides (30-100 bases long) may be synthesized, phosphorylated, purified and annealed under appropriate conditions using well known techniques. The ends of the oligonucleotides can be joined, for example, using T4 DNA ligase. This synthetic minigene, encoding the epitope polypeptide, can then be cloned into a desired expression vector.

Standard regulatory sequences well known to those skill in the art are preferably included in the vector to ensure expression in the target cells. Several vector elements are desirable: a promoter with a down-stream cloning site for minigene insertion; a polyadenylation signal for efficient transcription termination; an *E. coli* origin of replication; and an *E. coli* selectable marker (*e.g.* ampicillin or kanamycin resistance). Numerous promoters can be used for this purpose, *e.g.*, the human cytomegalovirus

(hCMV) promoter. See, e.g., U.S. Patent Nos. 5,580,859 and 5,589,466 for other suitable promoter sequences.

Additional vector modifications may be desired to optimize minigene expression and immunogenicity. In some cases, introns are required for efficient gene expression, and one or more synthetic or naturally-occurring introns could be incorporated into the transcribed region of the minigene. The inclusion of mRNA stabilization sequences and sequences for replication in mammalian cells may also be considered for increasing minigene expression.

Once an expression vector is selected, the minigene is cloned into the polylinker region downstream of the promoter. This plasmid is transformed into an appropriate *E. coli* strain, and DNA is prepared using standard techniques. The orientation and DNA sequence of the minigene, as well as all other elements included in the vector, are confirmed using restriction mapping and DNA sequence analysis. Bacterial cells harboring the correct plasmid can be stored as a master cell bank and a working cell bank.

In addition, immunostimulatory sequences (ISSs or CpGs) appear to play a role in the immunogenicity of DNA vaccines. These sequences may be included in the vector, outside the minigene coding sequence, if desired to enhance immunogenicity.

In some embodiments, a bi-cistronic expression vector which allows production of both the minigene-encoded epitopes and a second protein (included to enhance or decrease immunogenicity) can be used. Examples of proteins or polypeptides that could beneficially enhance the immune response if co-expressed include cytokines (e.g., IL-2, IL-12, GM-CSF), cytokine-inducing molecules (e.g., LeIF), costimulatory molecules, or for HTL responses, pan-DR binding proteins (PADRE™, Epimmune, San Diego, CA). Helper (HTL) epitopes can be joined to intracellular targeting signals and expressed separately from expressed CTL epitopes; this allows direction of the HTL epitopes to a cell compartment different than that of the CTL epitopes. If required, this could facilitate more efficient entry of HTL epitopes into the HLA class II pathway, thereby improving HTL induction. In contrast to HTL or CTL induction, specifically decreasing the immune response by co-expression of immunosuppressive molecules (e.g. TGF- β) may be beneficial in certain diseases.

Therapeutic quantities of plasmid DNA can be produced for example, by fermentation in *E. coli*, followed by purification. Aliquots from the working cell bank are used to inoculate growth medium, and grown to saturation in shaker flasks or a bioreactor

according to well known techniques. Plasmid DNA can be purified using standard bioseparation technologies such as solid phase anion-exchange resins supplied by QIAGEN, Inc. (Valencia, California). If required, supercoiled DNA can be isolated from the open circular and linear forms using gel electrophoresis or other methods.

5 Purified plasmid DNA can be prepared for injection using a variety of formulations. The simplest of these is reconstitution of lyophilized DNA in sterile phosphate-buffered saline (PBS). This approach, known as "naked DNA," is currently being used for intramuscular (IM) administration in clinical trials. To maximize the immunotherapeutic effects of minigene DNA vaccines, an alternative method for
10 formulating purified plasmid DNA may be desirable. A variety of methods have been described, and new techniques may become available. Cationic lipids, glycolipids, and fusogenic liposomes can also be used in the formulation (see, e.g., as described by WO 93/24640; Mannino & Gould-Fogerite, *BioTechniques* 6(7): 682 (1988); U.S. Pat No. 5,279,833; WO 91/06309; and Felgner, *et al.*, *Proc. Nat'l Acad. Sci. USA* 84:7413 (1987).
15 In addition, peptides and compounds referred to collectively as protective, interactive, non-condensing compounds (PINC) could also be complexed to purified plasmid DNA to influence variables such as stability, intramuscular dispersion, or trafficking to specific organs or cell types.

Target cell sensitization can be used as a functional assay for expression and HLA
20 class I presentation of minigene-encoded CTL epitopes. For example, the plasmid DNA is introduced into a mammalian cell line that is suitable as a target for standard CTL chromium release assays. The transfection method used will be dependent on the final formulation. Electroporation can be used for "naked" DNA, whereas cationic lipids allow direct *in vitro* transfection. A plasmid expressing green fluorescent protein (GFP) can be
25 co-transfected to allow enrichment of transfected cells using fluorescence activated cell sorting (FACS). These cells are then chromium-51 (^{51}Cr) labeled and used as target cells for epitope-specific CTL lines; cytolysis, detected by ^{51}Cr release, indicates both production of, and HLA presentation of, minigene-encoded CTL epitopes. Expression of
30 HTL epitopes may be evaluated in an analogous manner using assays to assess HTL activity.

In vivo immunogenicity is a second approach for functional testing of minigene DNA formulations. Transgenic mice expressing appropriate human HLA proteins are immunized with the DNA product. The dose and route of administration are formulation dependent (e.g., IM for DNA in PBS, intraperitoneal (IP) for lipid-complexed DNA).

Twenty-one days after immunization, splenocytes are harvested and restimulated for one week in the presence of peptides encoding each epitope being tested. Thereafter, for CTL effector cells, assays are conducted for cytolysis of peptide-loaded, ⁵¹Cr-labeled target cells using standard techniques. Lysis of target cells that were sensitized by HLA loaded
5 with peptide epitopes, corresponding to minigene-encoded epitopes, demonstrates DNA vaccine function for *in vivo* induction of CTLs. Immunogenicity of HTL epitopes is evaluated in transgenic mice in an analogous manner.

Alternatively, the nucleic acids can be administered using ballistic delivery as described, for instance, in U.S. Patent No. 5,204,253. Using this technique, particles
10 comprised solely of DNA are administered. In a further alternative embodiment, DNA can be adhered to particles, such as gold particles.

IV.K.2. Combinations of CTL Peptides with Helper Peptides

Vaccine compositions comprising the peptides of the present invention, or analogs
15 thereof, which have immunostimulatory activity may be modified to provide desired attributes, such as improved serum half-life, or to enhance immunogenicity.

For instance, the ability of a peptide to induce CTL activity can be enhanced by linking the peptide to a sequence which contains at least one epitope that is capable of inducing a T helper cell response. The use of T helper epitopes in conjunction with CTL
20 epitopes to enhance immunogenicity is illustrated, for example, in the co-pending applications U.S.S.N. 08/820,360, U.S.S.N. 08/197,484, and U.S.S.N. 08/464,234.

Particularly preferred CTL epitope/HTL epitope conjugates are linked by a spacer molecule. The spacer is typically comprised of relatively small, neutral molecules, such as amino acids or amino acid mimetics, which are substantially uncharged under
25 physiological conditions. The spacers are typically selected from, e.g., Ala, Gly, or other neutral spacers of nonpolar amino acids or neutral polar amino acids. It will be understood that the optionally present spacer need not be comprised of the same residues and thus may be a hetero- or homo-oligomer. When present, the spacer will usually be at least one or two residues, more usually three to six residues. Alternatively, the CTL
30 peptide may be linked to the T helper peptide without a spacer.

The CTL peptide epitope may be linked to the T helper peptide epitope either directly or via a spacer either at the amino or carboxy terminus of the CTL peptide. The amino terminus of either the immunogenic peptide or the T helper peptide may be acylated. The HTL peptide epitopes used in the invention can be modified in the same

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manner as CTL peptides. For instance, they may be modified to include D-amino acids or be conjugated to other molecules such as lipids, proteins, sugars and the like.

In certain embodiments, the T helper peptide is one that is recognized by T helper cells present in the majority of the population. This can be accomplished by selecting

5 amino acid sequences that bind to many, most, or all of the HLA class II molecules.

These are known as "loosely HLA-restricted" or "promiscuous" T helper sequences.

Examples of amino acid sequences that are promiscuous include sequences from antigens such as tetanus toxoid at positions 830-843 (QYIKANSKFIGITE), *Plasmodium*

falciparum CS protein at positions 378-398 (DIEKKIAKMEKASSVFNVVNS), and

10 Streptococcus 18kD protein at positions 116 (GAVDSILGGVATYGAA). Other examples include peptides bearing a DR 1-4-7 supermotif, or either of the DR3 motifs.

Alternatively, it is possible to prepare synthetic peptides capable of stimulating T helper lymphocytes, in a loosely HLA-restricted fashion, using amino acid sequences not found in nature (see, e.g., PCT publication WO 95/07707).

These synthetic compounds called Pan-DR-binding epitopes (e.g., PADRE™, Epimmune, Inc., San Diego, CA) are

15 designed to most preferably bind most HLA-DR (human HLA class II) molecules. For instance, a pan-DR-binding epitope peptide having the formula: aKXVWANTLKAa,

where "X" is either cyclohexylalanine, phenylalanine, or tyrosine, and "a" is either D-

alanine or L-alanine, has been found to bind to most HLA-DR alleles, and to stimulate the

20 response of T helper lymphocytes from most individuals, regardless of their HLA type.

An alternative of a pan-DR binding epitope comprises all "L" natural amino acids and can be provided in the form of nucleic acids that encode the epitope.

HTL peptide epitopes can also be modified to alter their biological properties. For example, peptides comprising HTL epitopes can contain D-amino acids to increase their

25 resistance to proteases and thus extend their serum half-life. Also, the epitope peptides of the invention can be conjugated to other molecules such as lipids, proteins or sugars, or

any other synthetic compounds, to increase their biological activity. Specifically, the T helper peptide can be conjugated to one or more palmitic acid chains at either the amino

or carboxyl termini.

30 In some embodiments it may be desirable to include in the pharmaceutical

compositions of the invention at least one component which primes cytotoxic T

lymphocytes. Lipids have been identified as agents capable of priming CTL *in vivo*

against viral antigens. For example, palmitic acid residues can be attached to the ε-and α-

amino groups of a lysine residue and then linked, e.g., via one or more linking residues such as Gly, Gly-Gly-, Ser, Ser-Ser, or the like, to an immunogenic peptide. The lipidated peptide can then be administered either directly in a micelle or particle, incorporated into a liposome, or emulsified in an adjuvant, e.g., incomplete Freund's
5 adjuvant. A particularly effective immunogen comprises palmitic acid attached to ε- and α- amino groups of Lys, which is attached via linkage, e.g., Ser-Ser, to the amino terminus of the immunogenic peptide.

As another example of lipid priming of CTL responses, *E. coli* lipoproteins, such as tripalmitoyl-S-glycerylcysteinylseryl- serine (P₃CSS) can be used to prime virus
10 specific CTL when covalently attached to an appropriate peptide (see, e.g., Deres, *et al.*, *Nature* 342:561, 1989). Peptides of the invention can be coupled to P₃CSS, for example, and the lipopeptide administered to an individual to specifically prime a CTL response to the target antigen. Moreover, because the induction of neutralizing antibodies can also be primed with P₃CSS-conjugated epitopes, two such compositions can be combined to more
15 effectively elicit both humoral and cell-mediated responses to infection.

As noted herein, additional amino acids can be added to the termini of a peptide to provide for ease of linking peptides one to another, for coupling to a carrier support or larger peptide, for modifying the physical or chemical properties of the peptide or oligopeptide, or the like. Amino acids such as tyrosine, cysteine, lysine, glutamic or aspartic acid, or the like, can be introduced at the C- or N-terminus of the peptide or
20 oligopeptide, particularly class I peptides. However, it is to be noted that modification at the carboxyl terminus of a CTL epitope may, in some cases, alter binding characteristics of the peptide. In addition, the peptide or oligopeptide sequences can differ from the natural sequence by being modified by terminal-NH₂ acylation, e.g., by alkanoyl (C₁-C₂₀)
25 or thioglycolyl acetylation, terminal-carboxyl amidation, e.g., ammonia, methylamine, etc. In some instances these modifications may provide sites for linking to a support or other molecule.

IV.L. Administration of Vaccines for Therapeutic or Prophylactic Purposes

The peptides of the present invention and pharmaceutical and vaccine compositions of the invention are useful for administration to mammals, particularly humans, to treat and/or prevent cancer. Vaccine compositions containing the peptides of the invention are administered to a cancer patient or to an individual susceptible to, or
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otherwise at risk for, cancer to elicit an immune response against TAAs and thus enhance the patient's own immune response capabilities. In therapeutic applications, peptide and/or nucleic acid compositions are administered to a patient in an amount sufficient to elicit an effective CTL and/or HTL response to the tumor antigen and to cure or at least
5 partially arrest or slow symptoms and/or complications. An amount adequate to accomplish this is defined as "therapeutically effective dose." Amounts effective for this use will depend on, *e.g.*, the particular composition administered, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician.

10 The vaccine compositions of the invention may also be used purely as prophylactic agents. Generally the dosage for an initial prophylactic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1000 µg and the higher value is about 10,000; 20,000; 30,000; or 50,000 µg. Dosage values for a human typically range from about 500 µg to about 50,000 µg per 70 kilogram patient. This is followed by boosting dosages of between about 1.0 µg to about 50,000 µg of peptide administered at defined intervals from about four weeks to six months after the initial administration of vaccine. The immunogenicity of the vaccine may be assessed by measuring the specific activity of CTL and HTL obtained from a sample of the patient's blood.

20 As noted above, peptides comprising CTL and/or HTL epitopes of the invention induce immune responses when presented by HLA molecules and contacted with a CTL or HTL specific for an epitope comprised by the peptide. The manner in which the peptide is contacted with the CTL or HTL is not critical to the invention. For instance, the peptide can be contacted with the CTL or HTL either *in vivo* or *in vitro*. If the
25 contacting occurs *in vivo*, the peptide itself can be administered to the patient, or other vehicles, *e.g.*, DNA vectors encoding one or more peptides, viral vectors encoding the peptide(s), liposomes and the like, can be used, as described herein.

When the peptide is contacted *in vitro*, the vaccinating agent can comprise a population of cells, *e.g.*, peptide-pulsed dendritic cells, or TAA-specific CTLs, which have been induced by pulsing antigen-presenting cells *in vitro* with the peptide. Such a cell population is subsequently administered to a patient in a therapeutically effective dose.

For pharmaceutical compositions, the immunogenic peptides of the invention, or DNA encoding them, are generally administered to an individual already diagnosed with

cancer. The peptides or DNA encoding them can be administered individually or as fusions of one or more peptide sequences.

For therapeutic use, administration should generally begin at the first diagnosis of cancer. This is followed by boosting doses until at least symptoms are substantially abated and for a period thereafter. The embodiment of the vaccine composition (i.e., including, but not limited to embodiments such as peptide cocktails, polyepitopic polypeptides, minigenes, or TAA-specific CTLs) delivered to the patient may vary according to the stage of the disease. For example, a vaccine comprising TAA-specific CTLs may be more efficacious in killing tumor cells in patients with advanced disease than alternative embodiments.

The vaccine compositions of the invention may also be used therapeutically in combination with treatments such as surgery. An example is a situation in which a patient has undergone surgery to remove a primary tumor and the vaccine is then used to slow or prevent recurrence and/or metastasis.

Where susceptible individuals, *e.g.*, individuals who may be diagnosed as being genetically pre-disposed to developing a particular type of tumor, are identified prior to diagnosis of cancer, the composition can be targeted to them, thus minimizing the need for administration to a larger population.

The dosage for an initial therapeutic immunization generally occurs in a unit dosage range where the lower value is about 1, 5, 50, 500, or 1,000 µg and the higher value is about 10,000; 20,000; 30,000; or 50,000 µg. Dosage values for a human typically range from about 500 µg to about 50,000 µg per 70 kilogram patient. Boosting dosages of between about 1.0 µg to about 50,000 µg of peptide pursuant to a boosting regimen over weeks to months may be administered depending upon the patient's response and condition as determined by measuring the specific activity of CTL and HTL obtained from the patient's blood. The peptides and compositions of the present invention may be employed in serious disease states, that is, life-threatening or potentially life threatening situations. In such cases, as a result of the minimal amounts of extraneous substances and the relative nontoxic nature of the peptides in preferred compositions of the invention, it is possible and may be felt desirable by the treating physician to administer substantial excesses of these peptide compositions relative to these stated dosage amounts.

Thus, for treatment of cancer, a representative dose is in the range disclosed above, namely where the lower value is about 1, 5, 50, 500, or 1,000 µg and the higher

value is about 10,000; 20,000; 30,000; or 50,000 µg, preferably from about 500 µg to about 50,000 µg per 70 kilogram patient. Initial doses followed by boosting doses at established intervals, *e.g.*, from four weeks to six months, may be required, possibly for a prolonged period of time to effectively immunize an individual. Administration should
5 continue until at least clinical symptoms or laboratory tests indicate that the tumor has been eliminated or that the tumor cell burden has been substantially reduced and for a period thereafter. The dosages, routes of administration, and dose schedules are adjusted in accordance with methodologies known in the art.

The pharmaceutical compositions for therapeutic treatment are intended for
10 parenteral, topical, oral, intrathecal, or local administration. Preferably, the pharmaceutical compositions are administered parentally, *e.g.*, intravenously, subcutaneously, intradermally, or intramuscularly. Thus, the invention provides compositions for parenteral administration which comprise a solution of the immunogenic peptides dissolved or suspended in an acceptable carrier, preferably an aqueous carrier. A
15 variety of aqueous carriers may be used, *e.g.*, water, buffered water, 0.8% saline, 0.3% glycine, hyaluronic acid and the like. These compositions may be sterilized by conventional, well known sterilization techniques, or may be sterile filtered. The resulting aqueous solutions may be packaged for use as is, or lyophilized, the lyophilized preparation being combined with a sterile solution prior to administration. The
20 compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents, wetting agents, preservatives, and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, *etc.*.

25 The concentration of peptides of the invention in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.1%, usually at or at least about 2% to as much as 20% to 50% or more by weight, and will be selected primarily by fluid volumes, viscosities, *etc.*, in accordance with the particular mode of administration selected.

A human unit dose form of the peptide composition is typically included in a
30 pharmaceutical composition that comprises a human unit dose of an acceptable carrier, preferably an aqueous carrier, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans (*see, e.g.*, Remington's Pharmaceutical Sciences, 17th Edition, A. Gennaro, Editor, Mack Publishing Co., Easton, Pennsylvania, 1985).

The peptides of the invention may also be administered via liposomes, which serve to target the peptides to a particular tissue, such as lymphoid tissue, or to target selectively to infected cells, as well as to increase the half-life of the peptide composition. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, 5 phospholipid dispersions, lamellar layers and the like. In these preparations, the peptide to be delivered is incorporated as part of a liposome, alone or in conjunction with a molecule which binds to a receptor prevalent among lymphoid cells, such as monoclonal antibodies which bind to the CD45 antigen, or with other therapeutic or immunogenic compositions. Thus, liposomes either filled or decorated with a desired peptide of the 10 invention can be directed to the site of lymphoid cells, where the liposomes then deliver the peptide compositions. Liposomes for use in accordance with the invention are formed from standard vesicle-forming lipids, which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally guided by consideration of, *e.g.*, liposome size, acid lability and stability of the 15 liposomes in the blood stream. A variety of methods are available for preparing liposomes, as described in, *e.g.*, Szoka, *et al.*, *Ann. Rev. Biophys. Bioeng.* 9:467 (1980), and U.S. Patent Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

For targeting cells of the immune system, a ligand to be incorporated into the liposome can include, *e.g.*, antibodies or fragments thereof specific for cell surface 20 determinants of the desired immune system cells. A liposome suspension containing a peptide may be administered intravenously, locally, topically, *etc.* in a dose which varies according to, *inter alia*, the manner of administration, the peptide being delivered, and the stage of the disease being treated.

For solid compositions, conventional nontoxic solid carriers may be used which 25 include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition is formed by incorporating any of the normally employed excipients, such as those carriers previously listed, and generally 10-95% of active ingredient, that is, one or more peptides 30 of the invention, and more preferably at a concentration of 25%-75%.

For aerosol administration, the immunogenic peptides are preferably supplied in finely divided form along with a surfactant and propellant. Typical percentages of peptides are 0.01%-20% by weight, preferably 1%-10%. The surfactant must, of course, be nontoxic, and preferably soluble in the propellant. Representative of such agents are

the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1%-20% by weight
5 of the composition, preferably 0.25-5%. The balance of the composition is ordinarily propellant. A carrier can also be included, as desired, as with, e.g., lecithin for intranasal delivery.

IV.M. Kits

10 The peptide and nucleic acid compositions of this invention can be provided in kit form together with instructions for vaccine administration. Typically the kit would include desired peptide compositions in a container, preferably in unit dosage form and instructions for administration. An alternative kit would include a minigene construct with desired nucleic acids of the invention in a container, preferably in unit dosage form
15 together with instructions for administration. Lymphokines such as IL-2 or IL-12 may also be included in the kit. Other kit components that may also be desirable include, for example, a sterile syringe, booster dosages, and other desired excipients.

The invention will be described in greater detail by way of specific examples.

10 The following examples are offered for illustrative purposes, and are not intended to limit
20 the invention in any manner. Those of skill in the art will readily recognize a variety of non-critical parameters that can be changed or modified to yield alternative embodiments in accordance with the invention.

V. EXAMPLES

25 The following examples illustrate identification, selection, and use of immunogenic Class I and Class II peptide epitopes for inclusion in vaccine compositions.

Example 1. HLA Class I and Class II Binding Assays

30 The following example of peptide binding to HLA molecules demonstrates quantification of binding affinities of HLA class I and class II peptides. Binding assays can be performed with peptides that are either motif-bearing or not motif-bearing.

Epstein-Barr virus (EBV)-transformed homozygous cell lines, fibroblasts, CIR, or 721.221-transfectants were used as sources of HLA class I molecules. These cells were maintained *in vitro* by culture in RPMI 1640 medium supplemented with 2mM L-

glutamine (GIBCO, Grand Island, NY), 50 μ M 2-ME, 100 μ g/ml of streptomycin, 100U/ml of penicillin (Irvine Scientific) and 10% heat-inactivated FCS (Irvine Scientific, Santa Ana, CA). Cells were grown in 225-cm² tissue culture flasks or, for large-scale cultures, in roller bottle apparatuses. The specific cell lines routinely used for purification 5 of MHC class I and class II molecules are listed in Table XXIV.

Cell lysates were prepared and HLA molecules purified in accordance with disclosed protocols (Sidney *et al.*, *Current Protocols in Immunology* 18.3.1 (1998); Sidney, *et al.*, *J. Immunol.* 154:247 (1995); Sette, *et al.*, *Mol. Immunol.* 31:813 (1994)). Briefly, cells were lysed at a concentration of 10⁸ cells/ml in 50 mM Tris-HCl, pH 8.5, 10 containing 1% Nonidet P-40 (Fluka Biochemika, Buchs, Switzerland), 150 mM NaCl, 5 mM EDTA, and 2 mM PMSF. Lysates were cleared of debris and nuclei by centrifugation at 15,000 x g for 30min.

HLA molecules were purified from lysates by affinity chromatography. Lysates prepared as above were passed twice through two pre-columns of inactivated Sepharose 15 CL4-B and protein A-Sepharose. Next, the lysate was passed over a column of Sepharose CL-4B beads coupled to an appropriate antibody. The antibodies used for the extraction of HLA from cell lysates are listed in Table XXV. The anti-HLA column was then washed with 10-column volumes of 10mM Tris-HCL, pH 8.0, in 1% NP-40, PBS, 2-column volumes of PBS, and 2-column volumes of PBS containing 0.4% n-octylglucoside. Finally, MHC molecules were eluted with 50mM diethylamine in 0.15M 20 NaCl containing 0.4% n-octylglucoside, pH 11.5. A 1/25 volume of 2.0M Tris, pH 6.8, was added to the eluate to reduce the pH to ~8.0. Eluates were then concentrated by centrifugation in Centriprep 30 concentrators at 2000 rpm (Amicon, Beverly, MA). Protein content was evaluated by a BCA protein assay (Pierce Chemical Co., Rockford, 25 IL) and confirmed by SDS-PAGE.

A detailed description of the protocol utilized to measure the binding of peptides to Class I and Class II MHC has been published (Sette *et al.*, *Mol. Immunol.* 31:813, 1994; Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998). Briefly, purified MHC molecules (5 to 500nM) 30 were incubated with various unlabeled peptide inhibitors and 1-10nM ¹²⁵I-radiolabeled probe peptides for 48h in PBS containing 0.05% Nonidet P-40 (NP40) (or 20% w/v digitonin for H-2 IA assays) in the presence of a protease inhibitor cocktail. The final concentrations of protease inhibitors (each from CalBioChem, La Jolla, CA) were 1 mM

PMSF, 1.3 nM 1,10 phenanthroline, 73 μ M pepstatin A, 8mM EDTA, 6mM N-ethylmaleimide (for Class II assays), and 200 μ M N alpha-p-tosyl-L-lysine chloromethyl ketone (TLCK). All assays were performed at pH 7.0 with the exception of DRB1*0301, which was performed at pH 4.5, and DRB1*1601 (DR2w21 β_1) and DRB4*0101

5 (DRw53), which were performed at pH 5.0. pH was adjusted as described elsewhere (see Sidney *et al.*, in *Current Protocols in Immunology*, Margulies, Ed., John Wiley & Sons, New York, Section 18.3, 1998).

Following incubation, MHC-peptide complexes were separated from free peptide by gel filtration on 7.8 mm x 15 cm TSK200 columns (TosoHaas 16215,

10 Montgomeryville, PA), eluted at 1.2 mls/min with PBS pH 6.5 containing 0.5% NP40 and 0.1% NaN₃. Because the large size of the radiolabeled peptide used for the DRB1*1501 (DR2w2 β_1) assay makes separation of bound from unbound peaks more difficult under these conditions, all DRB1*1501 (DR2w2 β_1) assays were performed using a 7.8mm x 30cm TSK2000 column eluted at 0.6 mls/min. The eluate from the TSK columns was
15 passed through a Beckman 170 radioisotope detector, and radioactivity was plotted and integrated using a Hewlett-Packard 3396A integrator, and the fraction of peptide bound was determined.

Radiolabeled peptides were iodinated using the chloramine-T method.

20 Representative radiolabeled probe peptides utilized in each assay, and its assay specific IC₅₀ nM, are summarized in Tables IV and V. Typically, in preliminary experiments, each MHC preparation was titered in the presence of fixed amounts of radiolabeled peptides to determine the concentration of HLA molecules necessary to bind 10-20% of the total radioactivity. All subsequent inhibition and direct binding assays were performed using these HLA concentrations.

25 Since under these conditions [label]<[HLA] and IC₅₀≥[HLA], the measured IC₅₀ values are reasonable approximations of the true K_D values. Peptide inhibitors are typically tested at concentrations ranging from 120 μ g/ml to 1.2 ng/ml, and are tested in two to four completely independent experiments. To allow comparison of the data obtained in different experiments, a relative binding figure is calculated for each peptide
30 by dividing the IC₅₀ of a positive control for inhibition by the IC₅₀ for each tested peptide (typically unlabeled versions of the radiolabeled probe peptide). For database purposes, and inter-experiment comparisons, relative binding values are compiled. These values can subsequently be converted back into IC₅₀ nM values by dividing the IC₅₀ nM of the

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positive controls for inhibition by the relative binding of the peptide of interest. This method of data compilation has proven to be the most accurate and consistent for comparing peptides that have been tested on different days, or with different lots of purified MHC.

5 Because the antibody used for HLA-DR purification (LB3.1) is α -chain specific, β_1 molecules are not separated from β_3 (and/or β_4 and β_5) molecules. The β_1 specificity of the binding assay is obvious in the cases of DRB1*0101 (DR1), DRB1*0802 (DR8w2), and DRB1*0803 (DR8w3), where no β_3 is expressed. It has also been demonstrated for DRB1*0301 (DR3) and DRB3*0101 (DR52a), DRB1*0401 (DR4w4), DRB1*0404 (DR4w14), DRB1*0405 (DR4w15), DRB1*1101 (DR5), DRB1*1201 (DR5w12), DRB1*1302 (DR6w19) and DRB1*0701 (DR7). The problem of β chain specificity for DRB1*1501 (DR2w2 β_1), DRB5*0101 (DR2w2 β_2), DRB1*1601 (DR2w21 β_1), DRB5*0201 (DR51Dw21), and DRB4*0101 (DRw53) assays is circumvented by the use of fibroblasts. Development and validation of assays with regard to DR β molecule 10 specificity have been described previously (*see, e.g.*, Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998).

15

Binding assays as outlined above may be used to analyze supermotif and/or motif-bearing epitopes as, for example, described in Example 2.

20 Example 2. Identification of HLA Supermotif- and Motif-Bearing CTL Candidate Epitopes

Vaccine compositions of the invention may include multiple epitopes that comprise multiple HLA supermotifs or motifs to achieve broad population coverage. This example illustrates the identification of supermotif- and motif-bearing epitopes for 25 the inclusion in such a vaccine composition. Calculation of population coverage is performed using the strategy described below.

Computer searches and algorithms for identification of supermotif and/or motif-bearing epitopes

30 The searches performed to identify the motif-bearing peptide sequences in Examples 2 and 5 employed protein sequence data for the tumor-associated antigens MAGE2/3.

Computer searches for epitopes bearing HLA Class I or Class II supermotifs or motifs were performed as follows. All translated protein sequences were analyzed using a text string search software program, e.g., MotifSearch 1.4 (D. Brown, San Diego) to identify potential peptide sequences containing appropriate HLA binding motifs;

5 alternative programs are readily produced in accordance with information in the art in view of the motif/supermotif disclosure herein. Furthermore, such calculations can be made mentally. Identified A2-, A3-, and DR-supermotif sequences were scored using polynomial algorithms to predict their capacity to bind to specific HLA-Class I or Class II molecules. These polynomial algorithms take into account both extended and refined

10 motifs (that is, to account for the impact of different amino acids at different positions), and are essentially based on the premise that the overall affinity (or ΔG) of peptide-HLA molecule interactions can be approximated as a linear polynomial function of the type:

$$\Delta G = a_{1i} \times a_{2i} \times a_{3i} \dots \times a_{ni}$$

where a_{ji} is a coefficient which represents the effect of the presence of a given amino acid (j) at a given position (i) along the sequence of a peptide of n amino acids. The crucial assumption of this method is that the effects at each position are essentially independent of each other (i.e., independent binding of individual side-chains). When residue j occurs at position i in the peptide, it is assumed to contribute a constant amount j_i to the free energy of binding of the peptide irrespective of the sequence of the rest of the peptide.

15 This assumption is justified by studies from our laboratories that demonstrated that peptides are bound to MHC and recognized by T cells in essentially an extended conformation (data omitted herein).

The method of derivation of specific algorithm coefficients has been described in Gulukota *et al.*, *J. Mol. Biol.* 267:1258-126, 1997; (see also Sidney *et al.*, *Human Immunol.* 45:79-93, 1996; and Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). Briefly, for all i positions, anchor and non-anchor alike, the geometric mean of the average relative binding (ARB) of all peptides carrying j is calculated relative to the remainder of the group, and used as the estimate of j_i . For Class II peptides, if multiple alignments are possible, only the highest scoring alignment is utilized, following an iterative procedure. To calculate an algorithm score of a given peptide in a test set, the ARB values corresponding to the sequence of the peptide are multiplied. If this product exceeds a chosen threshold, the peptide is predicted to bind. Appropriate thresholds are chosen as a function of the degree of stringency of prediction desired.

Selection of HLA-A2 supertype cross-reactive peptides

The complete protein sequences from MAGE2/3 were scanned, utilizing motif identification software, to identify 8-, 9-, 10-, and 11-mer sequences containing the HLA-A2-supermotif main anchor specificity.

A total of 285 HLA-A2 supermotif-positive sequences were identified within the MAGE2 and/or MAGE3 protein sequences. Of these, 137 of the corresponding peptides were synthesized and tested for the capacity to bind purified HLA-A*0201 molecules *in vitro* (HLA-A*0201 is considered a prototype A2 supertype molecule). Nineteen of the peptides bound A*0201 with IC₅₀ values ≤500 nM.

The 19 A*0201-binding peptides were subsequently tested for the capacity to bind to additional A2-supertype molecules (A*0202, A*0203, A*0206, and A*6802). As shown in Table XXVI, 17 of the 19 peptides were found to be A2-supertype cross-reactive binders, binding at least three of the five A2-supertype alleles tested. One of the peptides was selected for further evaluation.

Selection of HLA-A3 supermotif-bearing epitopes

The protein sequences scanned above are also examined for the presence of peptides with the HLA-A3-supermotif primary anchors using methodology similar to that performed to identify HLA-A2 supermotif-bearing epitopes.

Peptides corresponding to the supermotif-bearing sequences are then synthesized and tested for binding to HLA-A*0301 and HLA-A*1101 molecules, the two most prevalent A3-supertype alleles. The peptides that are found to bind one of the two alleles with binding affinities of ≤500 nM are then tested for binding cross-reactivity to the other common A3-supertype alleles (A*3101, A*3301, and A*6801) to identify those that can bind at least three of the five HLA-A3-supertype molecules tested.

Selection of HLA-B7 supermotif bearing epitopes

The same target antigen protein sequences are also analyzed to identify HLA-B7-supermotif-bearing sequences. The corresponding peptides are then synthesized and tested for binding to HLA-B*0702, the most common B7-supertype allele (*i.e.*, the prototype B7 supertype allele). Those peptides that bind B*0702 with IC₅₀ of ≤500 nM are then tested for binding to other common B7-supertype molecules (B*3501, B*5101,

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B*5301, and B*5401) to identify those peptides that are capable of binding to three or more of the five B7-supertype alleles tested.

Selection of A1 and A24 motif-bearing epitopes

5 To further increase population coverage, HLA-A1 and -A24 epitopes can also be incorporated into potential vaccine constructs. An analysis of the protein sequence data from the target antigens utilized above can also be performed to identify HLA-A1- and A24-motif-containing conserved sequences.

10 Example 3. Confirmation of Immunogenicity

Motif analysis and binding studies described in Example 2 identified seventeen potential epitopes for both MAGE2 and MAGE3. Four of the peptide are, however, identical in both MAGE2 and 3, and therefore do not represent distinct epitopes. A total of 13 peptides were selected for *in vitro* immunogenicity testing. Testing was performed
15 using the following methodology:

Target Cell Lines for Cellular Screening:

The .221A2.1 cell line, produced by transferring the HLA-A2.1 gene into the HLA-A, -B, -C null mutant human B-lymphoblastoid cell line 721.221, was used as the
20 peptide-loaded target to measure activity of HLA-A2.1-restricted CTL. The HLA-typed melanoma cell lines (624mel and 888mel) were obtained from Y. Kawakami and S. Rosenberg, National Cancer Institute, Bethesda, MD. The cell lines were maintained in RPMI-1640 medium supplemented with antibiotics, sodium pyruvate, nonessential amino acids and 10% (v/v) heat inactivated FCS. The melanoma cells were treated with
25 100U/ml IFN γ (Genzyme) for 48 hours at 37°C before use as targets in the ^{51}Cr release and *in situ* IFN γ assays.

Primary CTL Induction Cultures:

Generation of Dendritic Cells (DC): PBMCs were thawed in RPMI with 30 $\mu\text{g}/\text{ml}$ DNAse, washed twice and resuspended in complete medium (RPMI-1640 plus 5% AB
30 human serum, non-essential amino acids, sodium pyruvate, L-glutamine and penicillin/strpetomycin). The monocytes were purified by plating 10×10^6 PBMC/well in a 6-well plate. After 2 hours at 37°C, the non-adherent cells were removed by gently

shaking the plates and aspirating the supernatants. The wells were washed a total of three times with 3 ml RPMI to remove most of the non-adherent and loosely adherent cells. Three ml of complete medium containing 50 ng/ml of GM-CSF and 1,000 U/ml of IL-4 were then added to each well. DC were used for CTL induction cultures following 7 days of culture.

Induction of CTL with DC and Peptide: CD8+ T-cells were isolated by positive selection with Dynal immunomagnetic beads (Dynabeads® M-450) and the detach-a-bead® reagent. Typically about $200-250 \times 10^6$ PBMC were processed to obtain 24×10^6 CD8⁺ T-cells (enough for a 48-well plate culture). Briefly, the PBMCs were thawed in 10 RPMI with 30 μ g/ml DNase, washed once with PBS containing 1% human AB serum and resuspended in PBS/1% AB serum at a concentration of 20×10^6 cells/ml. The magnetic beads were washed 3 times with PBS/AB serum, added to the cells (140 μ l beads/ 20×10^6 cells) and incubated for 1 hour at 4°C with continuous mixing. The beads and cells were washed 4x with PBS/AB serum to remove the nonadherent cells and 15 resuspended at 100×10^6 cells/ml (based on the original cell number) in PBS/AB serum containing 100 μ l/ml detach-a-bead® reagent and 30 μ g/ml DNase. The mixture is incubated for 1 hour at room temperature with continuous mixing. The beads were washed again with PBS/AB/DNase to collect the CD8+ T-cells. The DC were collected and centrifuged at 1300 rpm for 5-7 minutes, washed once with PBS with 1% BSA, 20 counted and pulsed with 40 μ g/ml of peptide at a cell concentration of $1-2 \times 10^6$ /ml in the presence of 3 μ g/ml β_2 - microglobulin for 4 hours at 20°C. The DC were then irradiated (4,200 rads), washed 1 time with medium and counted again.

Setting up induction cultures: 0.25 ml cytokine-generated DC (@ 1×10^5 cells/ml) were co-cultured with 0.25ml of CD8+ T-cells (@ 2×10^6 cell/ml) in each well of 25 a 48-well plate in the presence of 10 ng/ml of IL-7. rHuman IL10 was added the next day at a final concentration of 10 ng/ml and rhuman IL2 was added 48 hours later at 10IU/ml.

Restimulation of the induction cultures with peptide-pulsed adherent cells: Seven and fourteen days after the primary induction the cells were restimulated with peptide-pulsed adherent cells. The PBMCS were thawed and washed twice with RPMI 30 and DNase. The cells were resuspended at 5×10^6 cells/ml and irradiated at ~4200 rads. The PBMCS were plated at 2×10^6 in 0.5ml complete medium per well and incubated for 2 hours at 37°C. The plates were washed twice with RPMI by tapping the plate gently to remove the nonadherent cells and the adherent cells pulsed with 10 μ g/ml of peptide in the presence of 3 μ g/ml β_2 microglobulin in 0.25ml RPMI/5%AB per well for 2 hours at

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37°C. Peptide solution from each well was aspirated and the wells were washed once with RPMI. Most of the media was aspirated from the induction cultures (CD8+ cells) and brought to 0.5 ml with fresh media. The cells were then transferred to the wells containing the peptide-pulsed adherent cells. Twenty four hours later rhuman IL10 was
5 added at a final concentration of 10ng/ml and rhuman IL2 was added the next day and again 2-3 days later at 50IU/ml (Tsai *et al.*, *Critical Reviews in Immunology* 18(1-2):65-75, 1998). Seven days later the cultures were assayed for CTL activity in a
10 ⁵¹Cr release assay. In some experiments the cultures were assayed for peptide-specific recognition in the in situ IFN γ ELISA at the time of the second restimulation followed by assay of endogenous recognition 7 days later. After expansion, activity was measured in both assays for a side by side comparison.

Measurement of CTL lytic activity by ⁵¹Cr release.

Seven days after the second restimulation, cytotoxicity was determined in a standard (5hr) ⁵¹Cr release assay by assaying individual wells at a single E:T. Peptide-pulsed targets were prepared by incubating the cells with 10 μ g/ml peptide overnight at
15 37°C.

Adherent target cells were removed from culture flasks with trypsin-EDTA. Target cells were labelled with 200 μ Ci of ⁵¹Cr sodium chromate (Dupont, Wilmington, DE) for 1 hour at 37°C. Labelled target cells are resuspended at 10⁶ per ml
20 and diluted 1:10 with K562 cells at a concentration of 3.3x10⁶/ml (an NK-sensitive erythroblastoma cell line used to reduce non-specific lysis). Target cells (100 μ l) and 100 μ l of effectors were plated in 96 well round-bottom plates and incubated for 5 hours at 37°C. At that time, 100 μ l of supernatant were collected from each well and percent lysis
25 was determined according to the formula: [(cpm of the test sample- cpm of the spontaneous ⁵¹Cr release sample)/(cpm of the maximal ⁵¹Cr release sample- cpm of the spontaneous ⁵¹Cr release sample)] x 100. Maximum and spontaneous release were determined by incubating the labelled targets with 1% Triton X-100 and media alone, respectively. A positive culture was defined as one in which the specific lysis (sample-background) was 10% or higher in the case of individual wells and was 15% or more at
30 the 2 highest E:T ratios when expanded cultures were assayed.

***In situ* Measurement of Human γ IFN Production as an Indicator of Peptide-specific and Endogenous Recognition**

5 Immulon 2 plates were coated with mouse anti-human IFN γ monoclonal antibody (4 μ g/ml 0.1M NaHCO₃, pH8.2) overnight at 4°C. The plates were washed with Ca²⁺, Mg²⁺-free PBS/0.05% Tween 20 and blocked with PBS/10% FCS for 2 hours, after which the CTLs (100 μ l/well) and targets (100 μ l/well) were added to each well, leaving empty wells for the standards and blanks (which received media only). The target cells, either peptide-pulsed or endogenous targets, were used at a concentration of 1x10⁶ cells/ml. The plates were incubated for 48 hours at 37°C with 5% CO₂.

10 Recombinant human IFN γ was added to the standard wells starting at 400 pg or 1200pg/100 μ l/well and the plate incubated for 2 hours at 37°C. The plates were washed and 100 μ l of biotinylated mouse anti-human IFN γ monoclonal antibody (4 μ g/ml in PBS/3%FCS/0.05% Tween 20) were added and incubated for 2 hours at room temperature. After washing again, 100 μ l HRP-streptavidin were added and incubated for 15 1 hour at room temperature. The plates were then washed 6x with wash buffer, 100 μ l/well developing solution (TMB 1:1) were added, and the plates allowed to develop for 5-15 minutes. The reaction was stopped with 50 μ l/well 1M H₃PO₄ and read at OD450. A culture was considered positive if it measured at least 50 pg of IFN γ /well above background and was twice the background level of expression.

20 **CTL Expansion.** Those cultures that demonstrated specific lytic activity against peptide-pulsed targets and/or tumor targets were expanded over a two week period with anti-CD3. Briefly, 5x10⁴ CD8+ cells were added to a T25 flask containing the following: 1x10⁶ irradiated (4,200 rad) PBMC (autologous or allogeneic) per ml, 2x10⁵ irradiated (8,000 rad) EBV- transformed cells per ml, and OKT3 (anti-CD3) at 25 30ng per ml in RPMI-1640 containing 10% (v/v) human AB serum, non-essential amino acids, sodium pyruvate, 25 μ M 2-mercaptoethanol, L-glutamine and penicillin/streptomycin. rHuman IL2 was added 24 hours later at a final concentration of 200IU/ml and every 3 days thereafter with fresh media at 50IU/ml. The cells were split if the cell concentration exceeded 1x10⁶/ml and the cultures were assayed between days 13 30 and 15 at E:T ratios of 30, 10, 3 and 1:1 in the ⁵¹Cr release assay or at 1x10⁶/ml in the *in situ* IFN γ assay using the same targets as before the expansion.

Immunogenicity of A2 supermotif-bearing peptides

The A2-supermotif cross-reactive binding peptides that were selected for further evaluation were tested in the cellular assay for the ability to induce peptide-specific CTL in normal individuals. In this analysis, a peptide was considered to be an epitope if it
 5 induced peptide-specific CTLs in at least 2 donors (unless otherwise noted) and if those CTLs also recognized the endogenously expressed peptide.

A total of 13 peptides were screened in the cellular assay and 9 peptides were shown to induce a response in PBMCs from at least 2 normal donors. CTLs to 5 of these peptides were also able to recognize endogenously expressed peptide (Table XXVII).
 10 Two of these peptide sequences, MAGE3.159 and MAGE3.160, overlap and, while both bind to 5 allele-specific HLA molecules, MAGE3.160 binds with a higher affinity to 4 of the 5 alleles. A IFN γ *in situ* ELISA of individual CTL cultures induced with MAGE3.159 showed that cells from five wells recognized the peptide-pulsed targets, and 2 of these wells also recognized the appropriate tumor target. Additionally, MAGE3.160
 15 induced a peptide-specific CTL response in 14 of 48 wells and 3 of these wells demonstrated endogenous recognition in the IFN γ assay.

MAGE3.112, MAGE2.157, and MAGE3.271 have also been identified as epitopes (see, e.g., Kawashima *et al.*, *Human Immunol.* 59:1-14, 1998; Visseren, *Int. J. Cancer* 73:125, 1997).
 20

*Evaluation of A*03/A11 immunogenicity*

HLA-A3 supermotif-bearing cross-reactive binding peptides are also evaluated for immunogenicity using methodology analogous for that used to evaluate the immunogenicity of the HLA-A2 supermotif peptides.
 25

Evaluation of B7 immunogenicity

Immunogenicity screening of the B7-supertype cross-reactive binding peptides identified in Example 2 are evaluated in a manner analogous to the evaluation of A2-and A3-supermotif-bearing peptides.
 30

Example 4. Implementation of the Extended Supermotif to Improve the Binding Capacity of Native Epitopes by Creating Analogs

HLA motifs and supermotifs (comprising primary and/or secondary residues) are useful in the identification and preparation of highly cross-reactive native peptides, as demonstrated herein. Moreover, the definition of HLA motifs and supermotifs also allows one to engineer highly cross-reactive epitopes by identifying residues within a native peptide sequence which can be analogued, or “fixed” to confer upon the peptide certain characteristics, *e.g.* greater cross-reactivity within the group of HLA molecules that comprise a supertype, and/or greater binding affinity for some or all of those HLA molecules. Examples of analog peptides that exhibit modulated binding affinity are set forth in this example.

Analoguing at Primary Anchor Residues

Peptide engineering strategies were implemented to further increase the cross-reactivity of the epitopes identified above. On the basis of the data disclosed, *e.g.*, in related and co-pending U.S.S.N 09/226,775, the main anchors of A2-supermotif-bearing peptides are altered, for example, to introduce a preferred L, I, V, or M at position 2, and I or V at the C-terminus.

Peptides that exhibit at least weak A*0201 binding (IC_{50} of 5000 nM or less), and carrying suboptimal anchor residues at either position 2, the C-terminal position, or both, can be fixed by introducing canonical substitutions (L at position 2 and V at the C-terminus). Those analogued peptides that show at least a three-fold increase in A*0201 binding and bind with an IC_{50} of 500 nM, or less were then tested for A2 cross-reactive binding along with their wild-type (WT) counterparts. Analogued peptides that bind at least three of the five A2 supertype alleles were then selected for cellular screening analysis.

Additionally, the selection of analogs for cellular screening analysis was further restricted by the capacity of the WT parent peptide to bind at least weakly, *i.e.*, bind at an IC_{50} of 5000nM or less, to three of more A2 supertype alleles. The rationale for this requirement is that the WT peptides must be present endogenously in sufficient quantity to be biologically relevant. Analogued peptides have been shown to have increased immunogenicity and cross-reactivity by T cells specific for the WT epitope (*see, e.g.*, Parkhurst *et al.*, *J. Immunol.* 157:2539, 1996; and Pogue *et al.*, *Proc. Natl. Acad. Sci. USA* 92:8166, 1995).

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In the cellular screening of these peptide analogs, it is important to demonstrate that analog-specific CTLs are also able to recognize the wild-type peptide and, when possible, tumor targets that endogenously express the epitope.

Of the 19 MAGE2/3-derived A*0201 binding peptides, 14 carried suboptimal anchor residues. Five analogs of two peptide epitopes were synthesized and tested for binding to HLA-A2 supertype molecules. MAGE3.112 analogs exhibited increased A*0201 binding affinity, but the parent peptide bound all 5 A2 supertype HLA molecules and significant improvement was not achieved. The MAGE3.220 analog, however, did demonstrate a 3-fold increase in A*0201 binding affinity and improved cross-reactive binding (Table XXII).

In addition, 24 of the 26 weak A*0201 binding peptides also met the criteria for analoguing and can be similarly analyzed for improved binding properties.

Those MAGE2/3 analogs that show improved binding relative to the wildtype peptide are evaluated in the cellular screening analysis as described in Example 3.

Using methodology similar to that used to develop HLA-A2 analogs, analogs of HLA-A3 and HLA-B7 supermotif-bearing epitopes may also be generated. For example, peptides binding at least weakly to 3/5 of the A3-supertype molecules may be engineered at primary anchor residues to possess a preferred residue (V, S, M, or A) at position 2. The analog peptides are then tested for the ability to bind A*03 and A*11 (prototype A3 supertype alleles). Those peptides that demonstrate ≤ 500 nM binding capacity are then tested for A3-supertype cross-reactivity. B7 supermotif-bearing peptides may, for example, be engineered to possess a preferred residue (V, I, L, or F) at the C-terminal primary anchor position, as demonstrated by Sidney *et al.* (*J. Immunol.* 157:3480-3490, 1996) and tested for binding to B7 supertype alleles.

25

Analoguing at Secondary Anchor Residues

Moreover, HLA supermotifs are of value in engineering highly cross-reactive peptides and/or peptides that bind HLA molecules with increased affinity by identifying particular residues at secondary anchor positions that are associated with such properties. For example, the binding capacity of a B7 supermotif-bearing peptide representing a discreet single amino acid substitution at position 1 can be analyzed. A peptide can, for example, be analogued to substitute L with F at position 1 and subsequently be evaluated for increased binding affinity/ and or increased cross-reactivity. This procedure will identify analogued peptides with modulated binding affinity.

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Engineered analogs with sufficiently improved binding capacity or cross-reactivity are tested for immunogenicity as above.

Other analoguing strategies

5 Another form of peptide analoguing, unrelated to the anchor positions, involves the substitution of a cysteine with α -amino butyric acid. Due to its chemical nature, cysteine has the propensity to form disulfide bridges and sufficiently alter the peptide structurally so as to reduce binding capacity. Substitution of α -amino butyric acid for cysteine not only alleviates this problem, but has been shown to improve binding and
10 crossbinding capabilities in some instances (see, e.g., the review by Sette *et al.*, In: Persistent Viral Infections, Eds. R. Ahmed and I. Chen, John Wiley & Sons, England, 1999).

In conclusion, these data demonstrate that by the use of even single amino acid substitutions, it is possible to increase the binding affinity and/or cross-reactivity of
15 peptide ligands for HLA supertype molecules.

Example 5. Identification of peptide epitope sequences with HLA-DR binding motifs

Peptide epitopes bearing an HLA class II supermotif or motif may also be identified as outlined below using methodology similar to that described in Examples 1-3.

Selection of HLA-DR-supermotif-bearing epitopes

To identify HLA class II HTL epitopes, the MAGE2/3 protein sequences were analyzed for the presence of sequences bearing an HLA-DR-motif or supermotif. Specifically, 15-mer sequences were selected comprising a DR-supermotif, further comprising a 9-mer core, and three-residue N- and C-terminal flanking regions (15 amino acids total).

Protocols for predicting peptide binding to DR molecules have been developed (Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). These protocols, specific for individual DR molecules, allow the scoring, and ranking, of 9-mer core regions. Each protocol not only scores peptide sequences for the presence of DR-supermotif primary anchors (i.e., at position 1 and position 6) within a 9-mer core, but additionally evaluates sequences for the presence of secondary anchors. Using allele specific selection tables (see, e.g., Southwood *et al.*, *ibid.*), it has been found that these protocols efficiently select

peptide sequences with a high probability of binding a particular DR molecule.

Additionally, it has been found that performing these protocols in tandem, specifically those for DR1, DR4w4, and DR7, can efficiently select DR cross-reactive peptides.

The MAGE2/3-derived peptides identified above were tested for their binding capacity for various common HLA-DR molecules. All peptides were initially tested for binding to the DR molecules in the primary panel: DR1, DR4w4, and DR7. Peptides binding at least 2 of these 3 DR molecules with an IC₅₀ value of 1000 nM or less, were then tested for binding to DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Peptides were considered to be cross-reactive DR supertype binders if they bound at an IC₅₀ value of 1000 nM or less to at least 5 of the 8 alleles tested.

Following the strategy outlined above, 97 DR supermotif-bearing sequences were identified within the MAGE2/3 protein sequences. Of those, 23 scored positive in 2 of the 3 combined DR 147 algorithms. These peptides were synthesized and tested for binding to HLA-DRB1*0101, DRB1*0401, DRB1*0701 with 13, 3, and 7 peptides binding ≤1000 nM, respectively. Of the 23 peptides tested for binding to these primary HLA molecules, 7 bound at least 2 of the 3 alleles (Table XXVIII).

These 7 peptides were then tested for binding to secondary DR supertype alleles: DRB5*0101, DRB1*1501, DRB1*1101, DRB1*0802, and DRB1*1302. Three of the peptides bound at least 5 of the 8 alleles tested, and occurred in distinct, non-overlapping regions (Table XXIX).

Selection of DR3 motif peptides

Because HLA-DR3 is an allele that is prevalent in Caucasian, Black, and Hispanic populations, DR3 binding capacity is an important criterion in the selection of HTL epitopes. However, data generated previously indicated that DR3 only rarely cross-reacts with other DR alleles (Sidney *et al.*, *J. Immunol.* 149:2634-2640, 1992; Geluk *et al.*, *J. Immunol.* 152:5742-5748, 1994; Southwood *et al.*, *J. Immunol.* 160:3363-3373, 1998). This is not entirely surprising in that the DR3 peptide-binding motif appears to be distinct from the specificity of most other DR alleles. For maximum efficiency in developing vaccine candidates it would be desirable for DR3 motifs to be clustered in proximity with DR supermotif regions. Thus, peptides shown to be candidates may also be assayed for their DR3 binding capacity. However, in view of the distinct binding specificity of the

DR3 motif, peptides binding only to DR3 can also be considered as candidates for inclusion in a vaccine formulation.

To efficiently identify peptides that bind DR3, the MAGE2/3 protein sequences were analyzed for conserved sequences carrying one of the two DR3 specific binding motifs (Table III) reported by Geluk *et al.* (*J. Immunol.* 152:5742-5748, 1994). Twenty-three motif-positive peptides were identified. The corresponding peptides were then synthesized and tested for the ability to bind DR3 with an affinity of \leq 1000 nM. Two peptides were identified that met this binding criterion (Table XXX), and thereby qualify as HLA class II high affinity binders.

The 2 DR3 binding peptides were then tested for binding to the DR supertype alleles (Table XXXI). Both DR3 binding peptides bound DRB1*1302 with an IC₅₀ of 269 nM, but neither was a DR supertype cross-reactive binder. Conversely, the DR supertype cross-reactive binding peptides were also tested for DR3 binding capacity, with no measurable DR3 binding observed.

In summary, 3 DR supertype cross-reactive binding peptides were identified from the MAGE2/3 protein sequences.

Similarly to the case of HLA class I motif-bearing peptides, the class II motif-bearing peptides may be analogued to improve affinity or cross-reactivity. For example, aspartic acid at position 4 of the 9-mer core sequence is an optimal residue for DR3 binding, and substitution for that residue may improve DR 3 binding.

Example 6. Immunogenicity of HTL epitopes

This example determines immunogenic DR supermotif- and DR3 motif-bearing epitopes among those identified using the methodology in Example 5. Immunogenicity of HTL epitopes are evaluated in a manner analogous to the determination of immunogenicity of CTL epitopes by assessing the ability to stimulate HTL responses and/or by using appropriate transgenic mouse models. Immunogenicity is determined by screening for: 1.) *in vitro* primary induction using normal PBMC or 2.) recall responses from cancer patient PBMCs.

Example 7. Calculation of phenotypic frequencies of HLA-supertypes in various ethnic backgrounds to determine breadth of population coverage

This example illustrates the assessment of the breadth of population coverage of a vaccine composition comprised of multiple epitopes comprising multiple supermotifs
5 and/or motifs.

In order to analyze population coverage, gene frequencies of HLA alleles were determined. Gene frequencies for each HLA allele were calculated from antigen or allele frequencies utilizing the binomial distribution formulae $gf=1-(\text{SQRT}(1-af))$ (see, e.g., Sidney *et al.*, *Human Immunol.* 45:79-93, 1996). To obtain overall phenotypic
10 frequencies, cumulative gene frequencies were calculated, and the cumulative antigen frequencies derived by the use of the inverse formula $[af=1-(1-Cgf)^2]$.

Where frequency data was not available at the level of DNA typing, correspondence to the serologically defined antigen frequencies was assumed. To obtain total potential supertype population coverage no linkage disequilibrium was assumed, and
15 only alleles confirmed to belong to each of the supertypes were included (minimal estimates). Estimates of total potential coverage achieved by inter-loci combinations were made by adding to the A coverage the proportion of the non-A covered population that could be expected to be covered by the B alleles considered (e.g., total=A+B*(1-A)). Confirmed members of the A3-like supertype are A3, A11, A31, A*3301, and A*6801.
20 Although the A3-like supertype may also include A34, A66, and A*7401, these alleles were not included in overall frequency calculations. Likewise, confirmed members of the A2-like supertype family are A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, and A*6901. Finally, the B7-like supertype-confirmed alleles are: B7, B*3501-03, B51, B*5301, B*5401, B*5501-2, B*5601, B*6701, and B*7801 (potentially
25 also B*1401, B*3504-06, B*4201, and B*5602).

Population coverage achieved by combining the A2-, A3- and B7-supertypes is approximately 86% in five major ethnic groups (see Table XXI). Coverage may be extended by including peptides bearing the A1 and A24 motifs. On average, A1 is present in 12% and A24 in 29% of the population across five different major ethnic
30 groups (Caucasian, North American Black, Chinese, Japanese, and Hispanic). Together, these alleles are represented with an average frequency of 39% in these same ethnic populations. The total coverage across the major ethnicities when A1 and A24 are combined with the coverage of the A2-, A3- and B7-supertype alleles is >95%. An

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analogous approach can be used to estimate population coverage achieved with combinations of class II motif-bearing epitopes.

Example 8. Recognition Of Generation Of Endogenous Processed Antigens After

5 Priming

This example determines that CTL induced by native or analogued peptide epitopes identified and selected as described in Examples 1-6 recognize endogenously synthesized, *i.e.*, native antigens, using a transgenic mouse model.

Effector cells isolated from transgenic mice that are immunized with peptide 10 epitopes (as described, e.g., in Wentworth et al., *Mol. Immunol.* 32:603, 1995), for example HLA-A2 supermotif-bearing epitopes, are re-stimulated *in vitro* using peptide-coated stimulator cells. Six days later, effector cells are assayed for cytotoxicity and the cell lines that contain peptide-specific cytotoxic activity are further re-stimulated. An additional six days later, these cell lines are tested for cytotoxic activity on ⁵¹Cr labeled 15 Jurkat-A2.1/K^b target cells in the absence or presence of peptide, and also tested on ⁵¹Cr labeled target cells bearing the endogenously synthesized antigen, *i.e.* cells that are stably transfected with TAA expression vectors.

The result will demonstrate that CTL lines obtained from animals primed with peptide epitope recognize endogenously synthesized antigen. The choice of transgenic 20 mouse model to be used for such an analysis depends upon the epitope(s) that is being evaluated. In addition to HLA-A*0201/K^b transgenic mice, several other transgenic mouse models including mice with human A11, which may also be used to evaluate A3 epitopes, and B7 alleles have been characterized and others (*e.g.*, transgenic mice for HLA-A1 and A24) are being developed. HLA-DR1 and HLA-DR3 mouse models have 25 also been developed, which may be used to evaluate HTL epitopes.

Example 9. Activity Of CTL-HTL Conjugated Epitopes In Transgenic Mice

This example illustrates the induction of CTLs and HTLs in transgenic mice by use of a tumor associated antigen CTL/HTL peptide conjugate whereby the vaccine 30 composition comprises peptides to be administered to a cancer patient. The peptide composition can comprise multiple CTL and/or HTL epitopes and further, can comprise epitopes selected from multiple-tumor associated antigens. The epitopes are identified using methodology as described in Examples 1-6. This analysis demonstrates the enhanced immunogenicity that can be achieved by inclusion of one or more HTL epitopes

in a vaccine composition. Such a peptide composition can comprise an HTL epitope conjugated to a preferred CTL epitope containing, for example, at least one CTL epitope selected from Tables XXII, XXVI, XXVII, or other analogs of that epitope. The HTL epitope is, for example, selected from Table XXXI. The peptides may be lipidated, if
5 desired.

Immunization procedures: Immunization of transgenic mice is performed as described (Alexander *et al.*, *J. Immunol.* 159:4753-4761, 1997). For example, A2/K^b mice, which are transgenic for the human HLA A2.1 allele and are useful for the assessment of the immunogenicity of HLA-A*0201 motif- or HLA-A2 supermotif-
10 bearing epitopes, are primed subcutaneously (base of the tail) with 0.1 ml of peptide conjugate formulated in saline, or DMSO/saline. Seven days after priming, splenocytes obtained from these animals are restimulated with syngenic irradiated LPS-activated lymphoblasts coated with peptide.

The target cells for peptide-specific cytotoxicity assays are Jurkat cells transfected with the HLA-A2.1/K^b chimeric gene (*e.g.*, Vitiello *et al.*, *J. Exp. Med.* 173:1007, 1991).

In vitro CTL activation: One week after priming, spleen cells (30x10⁶ cells/flask) are co-cultured at 37°C with syngeneic, irradiated (3000 rads), peptide coated lymphoblasts (10x10⁶ cells/flask) in 10 ml of culture medium/T25 flask. After six days, effector cells are harvested and assayed for cytotoxic activity.

Assay for cytotoxic activity: Target cells (1.0 to 1.5x10⁶) are incubated at 37°C in the presence of 200 µl of ⁵¹Cr. After 60 minutes, cells are washed three times and resuspended in medium. Peptide is added where required at a concentration of 1 µg/ml. For the assay, 10⁴ ⁵¹Cr-labeled target cells are added to different concentrations of effector cells (final volume of 200 µl) in U-bottom 96-well plates. After a 6 hour
20 incubation period at 37°C, a 0.1 ml aliquot of supernatant is removed from each well and radioactivity is determined in a Micromedic automatic gamma counter. The percent specific lysis is determined by the formula: percent specific release = 100 x (experimental release - spontaneous release)/(maximum release - spontaneous release). To facilitate comparison between separate CTL assays run under the same conditions, %
25 ⁵¹Cr release data is expressed as lytic units/10⁶ cells. One lytic unit is arbitrarily defined as the number of effector cells required to achieve 30% lysis of 10,000 target cells in a 6 hour ⁵¹Cr release assay. To obtain specific lytic units/10⁶, the lytic units/10⁶ obtained in the absence of peptide is subtracted from the lytic units/10⁶ obtained in the presence of peptide. For example, if 30% ⁵¹Cr release is obtained at the effector (E): target (T) ratio

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of 50:1 (i.e., 5×10^5 effector cells for 10,000 targets) in the absence of peptide and 5:1 (i.e., 5×10^4 effector cells for 10,000 targets) in the presence of peptide, the specific lytic units would be: $[(1/50,000)-(1/500,000)] \times 10^6 = 18$ LU.

The results are analyzed to assess the magnitude of the CTL responses of animals
 5 injected with the immunogenic CTL/HTL conjugate vaccine preparation. The magnitude and frequency of response can also be compared to the CTL response achieved using the CTL epitopes by themselves. Analyses similar to this may be performed to evaluate the immunogenicity of peptide conjugates containing multiple CTL epitopes and/or multiple HTL epitopes. In accordance with these procedures it is found that a CTL response is
 10 induced, and concomitantly that an HTL response is induced upon administration of such compositions.

Example 10. Selection of CTL and HTL epitopes for inclusion in a cancer vaccine.

This example illustrates the procedure for the selection of peptide epitopes for
 15 vaccine compositions of the invention. The peptides in the composition may be in the form of a nucleic acid sequence, either single or one or more sequences (*i.e.*, minigene) that encodes peptide(s), or may be single and/or polyepitopic peptides.

The following principles are utilized when selecting an array of epitopes for inclusion in a vaccine composition. Each of the following principles are balanced in
 20 order to make the selection.

1.) Epitopes are selected which, upon administration, mimic immune responses that have been observed to be correlated with tumor clearance. For HLA Class I this includes 3-4 epitopes that come from at least one TAA. For HLA Class II a similar rationale is employed; again 3-4 epitopes are selected from at least one TAA (*see e.g.*,
 25 Rosenberg *et al.*, *Science* 278:1447-1450). Epitopes from one TAA may be used in combination with epitopes from one or more additional TAAs to produce a vaccine that targets tumors with varying expression patterns of frequently-expressed TAAs as described, *e.g.*, in Example 15. The MAGE2/3 epitopes selected for inclusion are preferably conserved between the two proteins.

30 2.) Epitopes are selected that have the requisite binding affinity established to be correlated with immunogenicity: for HLA Class I an IC₅₀ of 500 nM or less, or for Class II an IC₅₀ of 1000 nM or less.

3.) Sufficient supermotif bearing peptides, or a sufficient array of allele-specific motif bearing peptides, are selected to give broad population coverage. For example, epitopes are selected to provide at least 80% population coverage. A Monte Carlo analysis, a statistical evaluation known in the art and discussed herein, can be
5 employed to assess breadth, or redundancy, of population coverage.

4.) When selecting epitopes from cancer-related antigens it is often preferred to select analogs because the patient may have developed tolerance to the native epitope. When selecting epitopes for infectious disease-related antigens it is preferable to select either native or analoged epitopes. Of relevance for infectious disease vaccines (but for
10 cancer-related vaccines as well), are epitopes referred to as "nested epitopes." Nested epitopes occur where at least two epitopes overlap in a given peptide sequence. A peptide comprising "transcendent nested epitopes" is a peptide that has both HLA class I and HLA class II epitopes in it.

When providing nested epitopes, a sequence that has the greatest number of epitopes per provided sequence is provided. A limitation on this principle is to avoid providing a peptide that is any longer than the amino terminus of the amino terminal epitope and the carboxyl terminus of the carboxyl terminal epitope in the peptide. When providing a longer peptide sequence, such as a sequence comprising nested epitopes, the sequence is screened in order to insure that it does not have pathological or other
20 deleterious biological properties.

5.) When creating a minigene, as disclosed in greater detail in Example 11, an objective is to generate the smallest peptide possible that encompasses the epitopes of interest. The principles employed are similar, if not the same as those employed when selecting a peptide comprising nested epitopes. Additionally, however, upon
25 determination of the nucleic acid sequence to be provided as a minigene, the peptide sequence encoded thereby is analyzed to determine whether any "junctional epitopes" have been created. A junctional epitope is a potential HLA binding epitope, as predicted, e.g., by motif analysis. Junctional epitopes are generally to be avoided because the recipient may bind to an HLA molecule and generate an immune response to that epitope,
30 which is not present in a native protein sequence. Of particular concern is a junctional epitope that is a "dominant epitope." A dominant epitope may lead to such a zealous response that immune responses to other epitopes are diminished or suppressed.

Peptide epitopes for inclusion in vaccine compositions are, for example, selected from those listed in Tables XXII, XXVI, XXVII, and XXXI. A vaccine composition

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comprised of selected peptides, when administered, is safe, efficacious, and elicits an immune response that results in tumor cell killing and reduction of tumor size or mass.

Example 11. Construction of Minigene Multi-Epitope DNA Plasmids

5 This example provides general guidance for the construction of a minigene expression plasmid. Minigene plasmids may, of course, contain various configurations of CTL and/or HTL epitopes or epitope analogs as described herein. Expression plasmids have been constructed and evaluated as described, for example, in co-pending U.S.S.N. 09/311,784 filed 5/13/99.

10 A minigene expression plasmid may include multiple CTL and HTL peptide epitopes. In the present example, HLA-A2, -A3, -B7 supermotif-bearing peptide epitopes and HLA-A1 and -A24 motif-bearing peptide epitopes are used in conjunction with DR supermotif-bearing epitopes and/or DR3 epitopes. Preferred epitopes are identified, for example, in Tables XXII, XXVI, XXVII, and XXXI. HLA class I supermotif or motif-bearing peptide epitopes derived from multiple TAAs are selected such that multiple supermotifs/motifs are represented to ensure broad population coverage. Similarly, HLA class II epitopes are selected from multiple tumor antigens to provide broad population coverage, *i.e.* both HLA DR-1-4-7 supermotif-bearing epitopes and HLA DR-3 motif-bearing epitopes are selected for inclusion in the minigene construct. The selected CTL
15 and HTL epitopes are then incorporated into a minigene for expression in an expression vector.
20

This example illustrates the methods to be used for construction of such a minigene-bearing expression plasmid. Other expression vectors that may be used for minigene compositions are available and known to those of skill in the art.

25 The minigene DNA plasmid contains a consensus Kozak sequence and a consensus murine kappa Ig-light chain signal sequence followed by CTL and/or HTL epitopes selected in accordance with principles disclosed herein. The sequence encodes an open reading frame fused to the Myc and His antibody epitope tag coded for by the pcDNA 3.1 Myc-His vector.

30 Overlapping oligonucleotides, for example eight oligonucleotides, averaging approximately 70 nucleotides in length with 15 nucleotide overlaps, are synthesized and HPLC-purified. The oligonucleotides encode the selected peptide epitopes as well as appropriate linker nucleotides, Kozak sequence, and signal sequence. The final multiepitope minigene is assembled by extending the overlapping oligonucleotides in

three sets of reactions using PCR. A Perkin/Elmer 9600 PCR machine is used and a total of 30 cycles are performed using the following conditions: 95°C for 15 sec, annealing temperature (5° below the lowest calculated Tm of each primer pair) for 30 sec, and 72°C for 1 min.

5 For the first PCR reaction, 5 µg of each of two oligonucleotides are annealed and extended: Oligonucleotides 1+2, 3+4, 5+6, and 7+8 are combined in 100 µl reactions containing *Pfu* polymerase buffer (1x= 10 mM KCL, 10 mM (NH₄)₂SO₄, 20 mM Tris-chloride, pH 8.75, 2 mM MgSO₄, 0.1% Triton X-100, 100 µg/ml BSA), 0.25 mM each dNTP, and 2.5 U of *Pfu* polymerase. The full-length dimer products are gel-purified, and
10 two reactions containing the product of 1+2 and 3+4, and the product of 5+6 and 7+8 are mixed, annealed, and extended for 10 cycles. Half of the two reactions are then mixed, and 5 cycles of annealing and extension carried out before flanking primers are added to amplify the full length product for 25 additional cycles. The full-length product is gel-purified and cloned into pCR-blunt (Invitrogen) and individual clones are screened by
15 sequencing.

Example 12. The plasmid construct and the degree to which it induces immunogenicity.

The degree to which the plasmid construct prepared using the methodology outlined in Example 11 is able to induce immunogenicity is evaluated through *in vivo* injections into mice and subsequent *in vitro* assessment of CTL and HTL activity, which are analysed using cytotoxicity and proliferation assays, respectively, as detailed e.g., in U.S.S.N. 09/311,784 filed 5/13/99 and Alexander *et al.*, *Immunity* 1:751-761, 1994.

Alternatively, plasmid constructs can be evaluated *in vitro* by testing for epitope presentation by APC following transduction or transfection of the APC with an epitope-expressing nucleic acid construct. Such a study determines “antigenicity” and allows the use of human APC. The assay determines the ability of the epitope to be presented by the APC in a context that is recognized by a T cell by quantifying the density of epitope-HLA class I complexes on the cell surface. Quantitation can be performed by directly measuring the amount of peptide eluted from the APC (see, e.g., Sijts *et al.*, *J. Immunol.* 156:683-692, 1996; Demotz *et al.*, *Nature* 342:682-684, 1989); or the number of peptide-HLA class I complexes can be estimated by measuring the amount of lysis or lymphokine release induced by infected or transfected target cells, and then determining the concentration of peptide necessary to obtain equivalent levels of lysis or lymphokine release (see, e.g., Kageyama *et al.*, *J. Immunol.* 154:567-576, 1995).

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To assess the capacity of the minigene construct (*e.g.*, a pMin minigene construct generated as described in U.S.S.N. 09/311,784) to induce CTLs *in vivo*, HLA-A11/K^b transgenic mice, for example, are immunized intramuscularly with 100 µg of naked cDNA. As a means of comparing the level of CTLs induced by cDNA immunization, a 5 control group of animals is also immunized with an actual peptide composition that comprises multiple epitopes synthesized as a single polypeptide as they would be encoded by the minigene.

Splenocytes from immunized animals are stimulated twice with each of the respective compositions (peptide epitopes encoded in the minigene or the polyepitopic peptide), then assayed for peptide-specific cytotoxic activity in a ⁵¹Cr release assay. The 10 results indicate the magnitude of the CTL response directed against the A3-restricted epitope, thus indicating the *in vivo* immunogenicity of the minigene vaccine and polyepitopic vaccine. It is, therefore, found that the minigene elicits immune responses directed toward the HLA-A3 supermotif peptide epitopes as does the polyepitopic peptide 15 vaccine. A similar analysis is also performed using other HLA-A2 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A2 and HLA-B7 motif or supermotif epitopes.

To assess the capacity of a class II epitope encoding minigene to induce HTLs *in vivo*, I-A^b restricted mice, for example, are immunized intramuscularly with 100 µg of 20 plasmid DNA. As a means of comparing the level of HTLs induced by DNA immunization, a group of control animals is also immunized with an actual peptide composition emulsified in complete Freund's adjuvant. CD4+ T cells, *i.e.* HTLs, are purified from splenocytes of immunized animals and stimulated with each of the respective compositions (peptides encoded in the minigene). The HTL response is 25 measured using a ³H-thymidine incorporation proliferation assay, (*see, e.g.*, Alexander et al. *Immunity* 1:751-761, 1994). The results indicate the magnitude of the HTL response, thus demonstrating the *in vivo* immunogenicity of the minigene.

DNA minigenes, constructed as described in Example 11, may also be evaluated 30 as a vaccine in combination with a boosting agent using a prime boost protocol. The boosting agent may consist of recombinant protein (*e.g.*, Barnett *et al.*, *Aids Res. and Human Retroviruses* 14, Supplement 3:S299-S309, 1998) or recombinant vaccinia, for example, expressing a minigene or DNA encoding the complete protein of interest (*see, e.g.*, Hanke *et al.*, *Vaccine* 16:439-445, 1998; Sedegah *et al.*, *Proc. Natl. Acad. Sci USA*

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95:7648-53, 1998; Hanke and McMichael, *Immunol. Letters* 66:177-181, 1999; and Robinson *et al.*, *Nature Med.* 5:526-34, 1999).

For example, the efficacy of the DNA minigene may be evaluated in transgenic mice. In this example, A2.1/K^b transgenic mice are immunized IM with 100 µg of the 5 DNA minigene encoding the immunogenic peptides. After an incubation period (ranging from 3-9 weeks), the mice are boosted IP with 10⁷ pfu/mouse of a recombinant vaccinia virus expressing the same sequence encoded by the DNA minigene. Control mice are immunized with 100 µg of DNA or recombinant vaccinia without the minigene sequence, or with DNA encoding the minigene, but without the vaccinia boost. After an additional 10 incubation period of two weeks, splenocytes from the mice are immediately assayed for peptide-specific activity in an ELISPOT assay. Additionally, splenocytes are stimulated *in vitro* with the A2-restricted peptide epitopes encoded in the minigene and recombinant vaccinia, then assayed for peptide-specific activity in an IFN-γ ELISA. It is found that the minigene utilized in a prime-boost mode elicits greater immune responses toward the 15 HLA-A2 supermotif peptides than with DNA alone. Such an analysis is also performed using other HLA-A11 and HLA-B7 transgenic mouse models to assess CTL induction by HLA-A3 and HLA-B7 motif or supermotif epitopes.

Example 13. Peptide Composition for Prophylactic Uses

Vaccine compositions of the present invention are used to prevent cancer in persons who are at risk for developing a tumor. For example, a polyepitopic peptide epitope composition (or a nucleic acid comprising the same) containing multiple CTL and HTL epitopes such as those selected in Examples 9 and/or 10, which are also selected to target greater than 80% of the population, is administered to an individual at risk for a 20 cancer, *e.g.*, melanoma. The composition is provided as a single polypeptide that encompasses multiple epitopes. The vaccine is administered in an aqueous carrier comprised of Freunds Incomplete Adjuvant. The dose of peptide for the initial immunization is from about 1 to about 50,000 µg, generally 100-5,000 µg, for a 70 kg patient. The initial administration of vaccine is followed by booster dosages at 4 weeks 25 followed by evaluation of the magnitude of the immune response in the patient, by techniques that determine the presence of epitope-specific CTL populations in a PBMC sample. Additional booster doses are administered as required. The composition is found 30 to be both safe and efficacious as a prophylaxis against cancer.

Alternatively, the polyepitopic peptide composition can be administered as a nucleic acid in accordance with methodologies known in the art and disclosed herein.

Example 14. Polyepitopic Vaccine Compositions Derived from Native TAA Sequences

5 A native TAA polyprotein sequence is screened, preferably using computer algorithms defined for each class I and/or class II supermotif or motif, to identify “relatively short” regions of the polyprotein that comprise multiple epitopes and is preferably less in length than an entire native antigen. This relatively short sequence that contains multiple distinct, even overlapping, epitopes is selected and used to generate a
10 minigene construct. The construct is engineered to express the peptide, which corresponds to the native protein sequence. The “relatively short” peptide is generally less than 1,000, 500, or 250 amino acids in length, often less than 100 amino acids in length, preferably less than 75 amino acids in length, and more preferably less than 50 amino acids in length. The protein sequence of the vaccine composition is selected
15 because it has maximal number of epitopes contained within the sequence, *i.e.*, it has a high concentration of epitopes. As noted herein, epitope motifs may be nested or overlapping (*i.e.*, frame shifted relative to one another). For example, with frame shifted overlapping epitopes, two 9-mer epitopes and one 10-mer epitope can be present in a 10 amino acid peptide. Such a vaccine composition is administered for therapeutic or
20 prophylactic purposes.

The vaccine composition will preferably include, for example, three CTL epitopes and at least one HTL epitope from TAAs. This polyepitopic native sequence is administered either as a peptide or as a nucleic acid sequence which encodes the peptide. Alternatively, an analog can be made of this native sequence, whereby one or more of the
25 epitopes comprise substitutions that alter the cross-reactivity and/or binding affinity properties of the polyepitopic peptide.

The embodiment of this example provides for the possibility that an as yet undiscovered aspect of immune system processing will apply to the native nested sequence and thereby facilitate the production of therapeutic or prophylactic immune response-inducing vaccine compositions. Additionally such an embodiment provides for the possibility of motif-bearing epitopes for an HLA makeup that is presently unknown. Furthermore, this embodiment (absent analogs) directs the immune response to multiple peptide sequences that are actually present in native TAAs thus avoiding the need to

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evaluate any junctional epitopes. Lastly, the embodiment provides an economy of scale when producing nucleic acid vaccine compositions.

Related to this embodiment, computer programs can be derived in accordance with principles in the art, which identify in a target sequence, the greatest number of epitopes per sequence length.

Example 15. Polyepitopic Vaccine Compositions Directed To Multiple Tumors

The MAGE2/3 peptide epitopes of the present invention are used in conjunction with peptide epitopes from other target tumor antigens to create a vaccine composition that is useful for the treatment of various types of tumors. For example, a set of TAA epitopes can be selected that allows the targeting of most common epithelial tumors (*see, e.g., Kawashima et al., Hum. Immunol. 59:1-14, 1998*). Such a composition includes epitopes from CEA, HER-2/neu, and MAGE2/3, all of which are expressed to appreciable degrees (20-60%) in frequently found tumors such as lung, breast, and gastrointestinal tumors.

The composition can be provided as a single polypeptide that incorporates the multiple epitopes from the various TAAs, or can be administered as a composition comprising one or more discrete epitopes. Alternatively, the vaccine can be administered as a minigene construct or as dendritic cells which have been loaded with the peptide epitopes *in vitro*.

Targeting multiple tumor antigens is also important to provide coverage of a large fraction of tumors of any particular type. A single TAA is rarely expressed in the majority of tumors of a given type. For example, approximately 50% of breast tumors express CEA, 20% express MAGE3, and 30% express HER-2/neu. Thus, the use of a single antigen for immunotherapy would offer only limited patient coverage. The combination of the three TAAs, however, would address approximately 70% of breast tumors. A vaccine composition comprising epitopes from multiple tumor antigens also reduces the potential for escape mutants due to loss of expression of an individual tumor antigen.

Example 16. Use of peptides to evaluate an immune response

Peptides of the invention may be used to analyze an immune response for the presence of specific CTL or HTL populations directed to a TAA. Such an analysis may be performed using multimeric complexes as described, *e.g., by Ogg et al., Science*

279:2103-2106, 1998 and Greten *et al.*, *Proc. Natl. Acad. Sci. USA* 95:7568-7573, 1998.

In the following example, peptides in accordance with the invention are used as a reagent for diagnostic or prognostic purposes, not as an immunogen.

In this example, highly sensitive human leukocyte antigen tetrameric complexes (“tetramers”) are used for a cross-sectional analysis of, for example, tumor-associated antigen HLA-A*0201-specific CTL frequencies from HLA A*0201-positive individuals at different stages of disease or following immunization using a TAA peptide containing an A*0201 motif. Tetrameric complexes are synthesized as described (Musey *et al.*, *N. Engl. J. Med.* 337:1267, 1997). Briefly, purified HLA heavy chain (A*0201 in this example) and β2-microglobulin are synthesized by means of a prokaryotic expression system. The heavy chain is modified by deletion of the transmembrane-cytosolic tail and COOH-terminal addition of a sequence containing a BirA enzymatic biotinylation site. The heavy chain, β2-microglobulin, and peptide are refolded by dilution. The 45-kD refolded product is isolated by fast protein liquid chromatography and then biotinylated by BirA in the presence of biotin (Sigma, St. Louis, Missouri), adenosine 5’triphosphate and magnesium. Streptavidin-phycoerythrin conjugate is added in a 1:4 molar ratio, and the tetrameric product is concentrated to 1 mg/ml. The resulting product is referred to as tetramer-phycoerythrin.

For the analysis of patient blood samples, approximately one million PBMCs are centrifuged at 300g for 5 minutes and resuspended in 50 µl of cold phosphate-buffered saline. Tri-color analysis is performed with the tetramer-phycoerythrin, along with anti-CD8-Tricolor, and anti-CD38. The PBMCs are incubated with tetramer and antibodies on ice for 30 to 60 min and then washed twice before formaldehyde fixation. Gates are applied to contain >99.98% of control samples. Controls for the tetramers include both A*0201-negative individuals and A*0201-positive uninfected donors. The percentage of cells stained with the tetramer is then determined by flow cytometry. The results indicate the number of cells in the PBMC sample that contain epitope-restricted CTLs, thereby readily indicating the extent of immune response to the TAA epitope, and thus the stage of tumor progression or exposure to a vaccine that elicits a protective or therapeutic response.

Example 17. Use of Peptide Epitopes to Evaluate Recall Responses

The peptide epitopes of the invention are used as reagents to evaluate T cell responses, such as acute or recall responses, in patients. Such an analysis may be performed on patients who are in remission, have a tumor, or who have been vaccinated
5 with a TAA vaccine.

For example, the class I restricted CTL response of persons who have been vaccinated may be analyzed. The vaccine may be any TAA vaccine. PBMC are collected from vaccinated individuals and HLA typed. Appropriate peptide epitopes of the invention that, optimally, bear supermotifs to provide cross-reactivity with multiple
10 HLA supertype family members, are then used for analysis of samples derived from individuals who bear that HLA type.

PBMC from vaccinated individuals are separated on Ficoll-Histopaque density gradients (Sigma Chemical Co., St. Louis, MO), washed three times in HBSS (GIBCO Laboratories), resuspended in RPMI-1640 (GIBCO Laboratories) supplemented with L-glutamine (2mM), penicillin (50U/ml), streptomycin (50 µg/ml), and Hepes (10mM)
15 containing 10% heat-inactivated human AB serum (complete RPMI) and plated using microculture formats. A synthetic peptide comprising an epitope of the invention is added at 10 µg/ml to each well and HBV core 128-140 epitope is added at 1 µg/ml to each well as a source of T cell help during the first week of stimulation.

In the microculture format, 4×10^5 PBMC are stimulated with peptide in 8 replicate cultures in 96-well round bottom plate in 100 µl/well of complete RPMI. On days 3 and 10, 100 µl of complete RPMI and 20 U/ml final concentration of rIL-2 are added to each well. On day 7 the cultures are transferred into a 96-well flat-bottom plate and restimulated with peptide, rIL-2 and 10^5 irradiated (3,000 rad) autologous feeder
25 cells. The cultures are tested for cytotoxic activity on day 14. A positive CTL response requires two or more of the eight replicate cultures to display greater than 10% specific ^{51}Cr release, based on comparison with uninfected control subjects as previously described (Rehermann, *et al.*, *Nature Med.* 2:1104,1108, 1996; Rehermann *et al.*, *J. Clin. Invest.* 97:1655-1665, 1996; and Rehermann *et al.* *J. Clin. Invest.* 98:1432-1440, 1996).

Target cell lines are autologous and allogeneic EBV-transformed B-LCL that are either purchased from the American Society for Histocompatibility and Immunogenetics (ASHI, Boston, MA) or established from the pool of patients as described (Guilhot, *et al.* *J. Virol.* 66:2670-2678, 1992).

Cytotoxicity assays are performed in the following manner. Target cells consist of either allogeneic HLA-matched or autologous EBV-transformed B lymphoblastoid cell line that are incubated overnight with the synthetic peptide epitope of the invention at 10 μM , and labeled with 100 μCi of ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 1 hour after which they are washed four times with HBSS.

Cytolytic activity is determined in a standard 4 hour, split-well ^{51}Cr release assay using U-bottomed 96 well plates containing 3,000 targets/well. Stimulated PBMC are tested at effector/target (E/T) ratios of 20-50:1 on day 14. Percent cytotoxicity is determined from the formula: $100 \times [(\text{experimental release-spontaneous release})/\text{maximum release-spontaneous release}]]$. Maximum release is determined by lysis of targets by detergent (2% Triton X-100; Sigma Chemical Co., St. Louis, MO). Spontaneous release is <25% of maximum release for all experiments.

The results of such an analysis indicate the extent to which HLA-restricted CTL populations have been stimulated by previous exposure to the TAA or TAA vaccine.

The class II restricted HTL responses may also be analyzed. Purified PBMC are cultured in a 96-well flat bottom plate at a density of 1.5×10^5 cells/well and are stimulated with 10 $\mu\text{g}/\text{ml}$ synthetic peptide, whole antigen, or PHA. Cells are routinely plated in replicates of 4-6 wells for each condition. After seven days of culture, the medium is removed and replaced with fresh medium containing 10U/ml IL-2. Two days later, 1 μCi ^{3}H -thymidine is added to each well and incubation is continued for an additional 18 hours. Cellular DNA is then harvested on glass fiber mats and analyzed for ^{3}H -thymidine incorporation. Antigen-specific T cell proliferation is calculated as the ratio of ^{3}H -thymidine incorporation in the presence of antigen divided by the ^{3}H -thymidine incorporation in the absence of antigen.

25

Example 18. Induction Of Specific CTL Response In Humans

A human clinical trial for an immunogenic composition comprising CTL and HTL epitopes of the invention is set up as an IND Phase I, dose escalation study. Such a trial is designed, for example, as follows:

30

A total of about 27 subjects are enrolled and divided into 3 groups:

Group I: 3 subjects are injected with placebo and 6 subjects are injected with 5 μg of peptide composition;

Group II: 3 subjects are injected with placebo and 6 subjects are injected with 50 µg peptide composition;

Group III: 3 subjects are injected with placebo and 6 subjects are injected with 500 µg of peptide composition.

- 5 After 4 weeks following the first injection, all subjects receive a booster inoculation at the same dosage. Additional booster inoculations can be administered on the same schedule.

10 The endpoints measured in this study relate to the safety and tolerability of the peptide composition as well as its immunogenicity. Cellular immune responses to the peptide composition are an index of the intrinsic activity of the peptide composition, and can therefore be viewed as a measure of biological efficacy. The following summarize 15 the clinical and laboratory data that relate to safety and efficacy endpoints.

Safety: The incidence of adverse events is monitored in the placebo and drug treatment group and assessed in terms of degree and reversibility.

- 15 Evaluation of Vaccine Efficacy: For evaluation of vaccine efficacy, subjects are bled before and after injection. Peripheral blood mononuclear cells are isolated from fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

The vaccine is found to be both safe and efficacious.

20

Example 19. Therapeutic Use in Cancer Patients

Evaluation of vaccine compositions are performed to validate the efficacy of the CTL-HTL peptide compositions in cancer patients. The main objectives of the trials are to determine an effective dose and regimen for inducing CTLs in cancer patients, to 25 establish the safety of inducing a CTL and HTL response in these patients, and to see to what extent activation of CTLs improves the clinical picture of cancer patients, as manifested by a reduction in tumor cell numbers. Such a study is designed, for example, as follows:

The studies are performed in multiple centers. The trial design is an open-label, 30 uncontrolled, dose escalation protocol wherein the peptide composition is administered as a single dose followed six weeks later by a single booster shot of the same dose. The dosages are 50, 500 and 5,000 micrograms per injection. Drug-associated adverse effects (severity and reversibility) are recorded.

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There are three patient groupings. The first group is injected with 50 micrograms of the peptide composition and the second and third groups with 500 and 5,000 micrograms of peptide composition, respectively. The patients within each group range in age from 21-65, include both males and females (unless the tumor is sex-specific, e.g., 5 breast or prostate cancer), and represent diverse ethnic backgrounds.

Example 20. Induction of CTL Responses Using a Prime Boost Protocol

A prime boost protocol similar in its underlying principle to that used to evaluate the efficacy of a DNA vaccine in transgenic mice, which was described in Example 12, 10 may also be used for the administration of the vaccine to humans. Such a vaccine regimen may include an initial administration of, for example, naked DNA followed by a boost using recombinant virus encoding the vaccine, or recombinant protein/polypeptide or a peptide mixture administered in an adjuvant.

For example, the initial immunization may be performed using an expression 15 vector, such as that constructed in Example 11, in the form of naked nucleic acid administered IM (or SC or ID) in the amounts of 0.5-5 mg at multiple sites. The nucleic acid (0.1 to 1000 μ g) can also be administered using a gene gun. Following an incubation period of 3-4 weeks, a booster dose is then administered. The booster can be recombinant fowlpox virus administered at a dose of $5\text{-}10^7$ to 5×10^9 pfu. An alternative recombinant 20 virus, such as an MVA, canarypox, adenovirus, or adeno-associated virus, can also be used for the booster, or the polyepitopic protein or a mixture of the peptides can be administered. For evaluation of vaccine efficacy, patient blood samples will be obtained before immunization as well as at intervals following administration of the initial vaccine and booster doses of the vaccine. Peripheral blood mononuclear cells are isolated from 25 fresh heparinized blood by Ficoll-Hypaque density gradient centrifugation, aliquoted in freezing media and stored frozen. Samples are assayed for CTL and HTL activity.

Analysis of the results will indicate that a magnitude of response sufficient to achieve protective immunity against cancer is generated.

30 Example 21. Administration of Vaccine Compositions Using Dendritic Cells

Vaccines comprising peptide epitopes of the invention may be administered using dendritic cells. In this example, the immunogenic peptide epitopes are used to elicit a CTL and/or HTL response *ex vivo*.

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Ex vivo CTL or HTL responses to a particular tumor-associated antigen are induced by incubating in tissue culture the patient's, or genetically compatible, CTL or HTL precursor cells together with a source of antigen-presenting cells (APC), such as dendritic cells, and the appropriate immunogenic peptides. After an appropriate 5 incubation time (typically about 7-28 days), in which the precursor cells are activated and expanded into effector cells, the cells are infused back into the patient, where they will destroy (CTL) or facilitate destruction (HTL) of their specific target cells, *i.e.*, tumor cells.

Alternatively, the peptide-pulsed dendritic cells may be administered to the patient 10 to stimulate a CTL response *in vivo*. In this method, dendritic cells are isolated as described in Example 3. The dendritic cell population is expanded and pulsed with a vaccine comprising peptide CTL and HTL epitopes of the invention. The dendritic cells are infused back into the patient to elicit CTL and HTL responses *in vivo*. The induced 15 CTL and HTL then destroy (CTL) or facilitate destruction (HTL) of the specific target tumor cells that bear the proteins from which the epitopes in the vaccine are derived.

Example 22. Alternative Method of Identifying Motif-Bearing Peptides

Another way of identifying motif-bearing peptides is to elute them from cells bearing defined MHC molecules. For example, EBV transformed B cell lines used for 20 tissue typing, have been extensively characterized to determine which HLA molecules they express. In certain cases these cells express only a single type of HLA molecule. These cells can then be infected with a pathogenic organism or transfected with nucleic acids that express the tumor antigen of interest. Thereafter, peptides produced by 25 endogenous antigen processing of peptides produced consequent to infection (or as a result of transfection) will bind to HLA molecules within the cell and be transported and displayed on the cell surface.

The peptides are then eluted from the HLA molecules by exposure to mild acid conditions and their amino acid sequence determined, *e.g.*, by mass spectral analysis (*e.g.*, Kubo *et al.*, *J. Immunol.* 152:3913, 1994). Because, as disclosed herein, the majority of 30 peptides that bind a particular HLA molecule are motif-bearing, this is an alternative modality for obtaining the motif-bearing peptides correlated with the particular HLA molecule expressed on the cell.

Alternatively, cell lines that do not express any endogenous HLA molecules can be transfected with an expression construct encoding a single HLA allele. These cells

may then be used as described, *i.e.*, they may be infected with a pathogenic organism or transfected with nucleic acid encoding an antigen of interest to isolate peptides corresponding to the pathogen or antigen of interest that have been presented on the cell surface. Peptides obtained from such an analysis will bear motif(s) that correspond to binding to the single HLA allele that is expressed in the cell.

As appreciated by one in the art, one can perform a similar analysis on a cell bearing more than one HLA allele and subsequently determine peptides specific for each HLA allele expressed. Moreover, one of skill would also recognize that means other than infection or transfection, such as loading with a protein antigen, can be used to provide a source of antigen to the cell.

The above examples are provided to illustrate the invention but not to limit its scope. For example, the human terminology for the Major Histocompatibility Complex, namely HLA, is used throughout this document. It is to be appreciated that these principles can be extended to other species as well. Thus, other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent application cited herein are hereby incorporated by reference for all purposes.

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TABLE I

SUPERMOTIFS	POSITION 2 (Primary Anchor)	POSITION 3 (Primary Anchor)	POSITION C Terminus (Primary Anchor)
A1	T <i>L</i> VMS		F WY
A2	L <i>I</i> VMA <i>T</i> <i>Q</i>		I VMATL
A3	V <i>S</i> MATL <i>I</i>		R K
A24	Y <i>F</i> WIVLMT		F IYWLM
B7	P		V ILFMWYA
B27	R HK		F YLWMIVA
B44	E D		F WYLIIMVA
B58	A TS		F WYLIIVMA
B62	Q LIVMP		F WYMIIVLA
<hr/>			
MOTIFS			
A1	T SM		Y
A1		D EAS	Y
A2.1	L MVQIAT		V LIMAT
A3	L MVISATFCGD		K YRHFA
A11	V TMLISAGNCDF		K RYH
A24	Y FWM		F LIW
A*3101	M VTALIS		R K
A*3301	M VALFIST		R K
A*6801	A VTMSLI		R K
B*0702	P		L MFWYAIIV
B*3501	P		L MFWYIIVA
B51	P		L IVFWYAM
B*5301	P		I MFWYALV
B*5401	P		A TIVLMFWY

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

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TABLE Ia

SUPERMOTIFS	POSITION	POSITION	POSITION
	2 (Primary Anchor)	3 (Primary Anchor)	C Terminus (Primary Anchor)
A1	TILVMS		FWY
A2	<i>VQAT</i>		VLIMAT
A3	VSMATLI		RK
A24	YFWIVLMT		FIYWLM
B7	P		VILFMWYA
B27	RHK		FYLWMIVA
B58	ATS		FWYLVIMA
B62	QLIVMP		FWYMICLA
<hr/>			
MOTIFS			
A1	TSM		Y
A1		DEAS	Y
A2.1	<i>VQAT*</i>		VLIMAT
A3.2	LMVISATFCGD		KYRHFA
A11	VTMLISAGNCDF		KRHY
A24	YFW		FLIW

*If 2 is V, or Q, the C-term is not L

Bolded residues are preferred, italicized residues are less preferred: A peptide is considered motif-bearing if it has primary anchors at each primary anchor position for a motif or supermotif as specified in the above table.

TABLE I

		POSITION								
		1	2	3	4	5	6	7	8	C-terminus
SUPERMOTIFS										
A1										$\frac{1}{\circ}$ Anchor FWY
A2										$\frac{1}{\circ}$ Anchor LIVMAT
A3	preferred				$\frac{1}{\circ}$ Anchor VSMATL	YFW (4/5)			YFW (3/5)	YFW (4/5)
	deleterious									P (4/5)
A24										$\frac{1}{\circ}$ Anchor FYWLM
B7	preferred	FWY (5/5)	LIVM (3/5)		$\frac{1}{\circ}$ Anchor P	FWY (4/5)			FWY (3/5)	$\frac{1}{\circ}$ Anchor VILFMWYA
	deleterious	DE (3/5); P(5/5); G(4/5); A(3/5); QN (3/5)					DE (3/5)	G (4/5)	QN (4/5)	DE (4/5)
B27										$\frac{1}{\circ}$ Anchor FYLMVIA
B44										$\frac{1}{\circ}$ Anchor FWYLIMVA
B58										$\frac{1}{\circ}$ Anchor FWYLIIVMA
B62										$\frac{1}{\circ}$ Anchor FWYMIIVLA

POSITION								
POSITION								
MOTIFS								
1	2	3	4	5	6	7	8	C-terminus
A1 9-mer	preferred	GFYW	<u>1°Anchor</u> STM	DEA	YFW	P	DEQN	YFW
deleterious	DE		RHKLIVM P	A	G	A		<u>1°Anchor</u> Y
A1 9-mer	preferred	GRHK	ASTCLIV M	<u>1°Anchor</u> DEAS	GSTC	ASTC	LIVM	DE
deleterious	A	RHKDPEY	FW	DE	PQN	RHK	PG	GP

		POSITION									
		[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	C-terminus
A1 10-mer	preferred	YFW	<u>1°Anchor</u> STM	DEAQN	A	YFWQN		PASTC	GDE	P	<u>1°Anchor</u> Y
deleterious	GP			RHKGLIV M	DE	RHK	QNA	RHKYFW	RHK	A	
<hr/>											
A1 10-mer	preferred	YFW	STCLIVM	<u>1°Anchor</u> DEAS	A	YFW		PG	G	YFW	<u>1°Anchor</u> Y
deleterious	RHK		RHKDEPY FW		P	G		PRHK	QN		
<hr/>											
A2.1 9-mer	preferred	YFW	<u>1°Anchor</u> LMIVQAT	YFW	STC	YFW		A	P	<u>1°Anchor</u> VLMAT	
deleterious	DEP			DERKH		RKH		DERKH			
<hr/>											
A2.1 10-mer	preferred	AYFW	<u>1°Anchor</u> LMIVQAT	LVM	G		G		FYWL VIM	<u>1°Anchor</u> VLMAT	
deleterious	DEP			DE	RKHA	P		RKH	DERK	RKHL	H

		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus
A3	preferred	RHK	$\frac{1^{\circ}\text{Anchor}}{\text{LMVISAT}}$		YFW	PRHKYFW	A	YFW	P	$\frac{1^{\circ}\text{Anchor}}{\text{KYRHF}A}$
	deleterious	DEP	FCGD							
			DE							
A11	preferred	A	$\frac{1^{\circ}\text{Anchor}}{\text{VTLMISA}}$		YFW	YFW	A	YFW	P	$\frac{1^{\circ}\text{Anchor}}{\text{KRYH}}$
	deleterious	DEP	GNCDF							
			A							
A24 9-mer	preferred	YFWRHK	$\frac{1^{\circ}\text{Anchor}}{\text{YFWWM}}$		STC		YFW		YFW	$\frac{1^{\circ}\text{Anchor}}{\text{FLJW}}$
	deleterious	DEG	DE		G		QNP		DERHK	G
			AQN							
A24 10-mer	preferred		$\frac{1^{\circ}\text{Anchor}}{\text{YFWWM}}$		P		YFWP		P	$\frac{1^{\circ}\text{Anchor}}{\text{FLIW}}$
	deleterious		GDE		QN		RHK		DE	A
			DIA							

		POSITION									
		[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]	C-terminus or
A3101	preferred	RHK	<u>1°Anchor</u> MVTALIS	YFW	P		YFW	YFW	AP		C-terminus 1°Anchor RK
deleterious	DEP			DE		ADE	DE	DE	DE		
A3301	preferred		<u>1°Anchor</u> MVALFIS T	YFW				AYFW			1°Anchor RK
deleterious	GP			DE							
A6801	preferred	YFWSTC	<u>1°Anchor</u> AVTMSLJ				YFWLIV M	YFW	P		1°Anchor RK
deleterious	GP			DEG			RHK			A	
B0702	preferred	RHKFWY	<u>1°Anchor</u> P	RHK			RHK	RHK	PA		1°Anchor LMFWYAIW
deleterious	DEQNP			DEP	DE	GDE		QN	DE		
B3501	preferred	FWYLIVM	<u>1°Anchor</u> P		FWY				FWY		1°Anchor 1.MFWYIWA
deleterious	AGP				G	G					

		POSITION								
		1	2	3	4	5	6	7	8	9 or C-terminus
B51	preferred	LIVMF ^{WY}	^{1°Anchor} <i>P</i>	FW ^Y	STC	FW ^Y		G	FW ^Y	^{1°Anchor} <i>LIVFWYAM</i>
deleterious		AGPDERHKSTC				DE	G	DEQN	GDE	
B5301	preferred	LIVMF ^{WY}	^{1°Anchor} <i>P</i>	FW ^Y	STC	FW ^Y		LIVMF ^{WY}	FW ^Y	^{1°Anchor} <i>IMFWYALW</i>
deleterious		AGPQN					G	RHKQN	DE	
B5401	preferred	FW ^Y	^{1°Anchor} <i>P</i>	FWYLIVM		LIVM		ALIVM	FWYAP	^{1°Anchor} <i>ATIVLMFW</i>
deleterious		GPQNDE		GDESTC		RHKDE	DE	QNDGE	DE	

Italicized residues indicate less preferred or “tolerated” residues.
The information in Table II is specific for 9-mers unless otherwise specified.

TABLE III

<u>MOTIFS</u>	<u>[1° anchor 1]</u>	<u>[2]</u>	<u>[3]</u>	<u>[4]</u>	<u>POSITION</u>	<u>[1° anchor 6]</u>	<u>[7]</u>	<u>[8]</u>	<u>[9]</u>
DR4 preferred	FM YLIW	M	T						
deleterious									
DR1 preferred	MFLIW W	C	CH		PAMQ				
deleterious					FD	CWD			
DR7 preferred	MFLIW W	M	W	A			IVMSACTPL	M	IVM
deleterious	C			G			GRD	N	G
DR Supermotif	MFLIW W				VMSTACPLI				
<u>DR3 MOTIFS</u>	<u>[1° anchor 1]</u>	<u>[2]</u>	<u>[3]</u>	<u>[4]</u>	<u>[1° anchor 4]</u>	<u>[5]</u>	<u>[6]</u>		
motif a preferred	LIVMFY				D				
motif b preferred	LIVMFAY						DNQUEST		
							KRH		

Italicized residues indicate less preferred or "tolerated" residues.

Table IV. HLA Class I Standard Peptide Binding Affinity.

ALLELE	STANDARD PEPTIDE	SEQUENCE	STANDARD BINDING AFFINITY (nM)
A*0101	944.02	YLEPAIAKY	25
A*0201	941.01	FLPSDYFPSV	5.0
A*0202	941.01	FLPSDYFPSV	4.3
A*0203	941.01	FLPSDYFPSV	10
A*0205	941.01	FLPSDYFPSV	4.3
A*0206	941.01	FLPSDYFPSV	3.7
A*0207	941.01	FLPSDYFPSV	23
A*6802	1072.34	YVIKVSARV	8.0
A*0301	941.12	KVFPYALINK	11
A*1101	940.06	AVDLYHFLK	6.0
A*3101	941.12	KVFPYALINK	18
A*3301	1083.02	STLPETYVVRR	29
A*6801	941.12	KVFPYALINK	8.0
A*2402	979.02	AYIDNYNKF	12
B*0702	1075.23	APRTLWYLL	5.5
B*3501	1021.05	FPPKYAAAF	7.2
B51	1021.05	FPPKYAAAF	5.5
B*5301	1021.05	FPPKYAAAF	9.3
B*5401	1021.05	FPPKYAAAF	10

Table V. HLA Class II Standard Peptide Binding Affinity.

Allele	Nomenclature	Standard Peptide	Sequence	Binding Affinity (nM)
DRB1*0101	DR1	515.01	PKYVKQNTLKLAT	5.0
DRB1*0301	DR3	829.02	YKTIAFDEEARR	300
DRB1*0401	DR4w4	515.01	PKYVKQNTLKLAT	45
DRB1*0404	DR4w14	717.01	YARFQSQTTLKQKT	50
DRB1*0405	DR4w15	717.01	YARFQSQTTLKQKT	38
DRB1*0701	DR7	553.01	QYIKANSKFIGITE	25
DRB1*0802	DR8w2	553.01	QYIKANSKFIGITE	49
DRB1*0803	DR8w3	553.01	QYIKANSKFIGITE	1600
DRB1*0901	DR9	553.01	QYIKANSKFIGITE	75
DRB1*1101	DR5w11	553.01	QYIKANSKFIGITE	20
DRB1*1201	DR5w12	1200.05	EALIHQLKINPYVLS	298
DRB1*1302	DR6w19	650.22	QYIKANAKFIGITE	3.5
DRB1*1501	DR2w2 β 1	507.02	GRTQDENPVVHFFKNIV TPRTPPP	9.1
DRB3*0101	DR52a	511	NGQIGNDPNRDIL	470
DRB4*0101	DRw53	717.01	YARFQSQTTLKQKT	58
DRB5*0101	DR2w2 β 2	553.01	QYIKANSKFIGITE	20

The "Nomenclature" column lists the allelic designations used in Tables XIX and XX.

Table VI

HLA-supertype	Allele-specific HLA-supertype members	
	Verified ^a	Predicted ^b
A1	A*0101, A*2501, A*2601, A*2602, A*3201	A*0102, A*2604, A*3601, A*4301, A*8001
A2	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*0209, A*0214, A*6802, A*6901	A*0208, A*0210, A*0211, A*0212, A*0213
A3	A*0301, A*1101, A*3101, A*3301, A*6801	A*0302, A*1102, A*2603, A*3302, A*3303, A*3401, A*3402, A*6601, A*6602, A*7401
A24	A*2301, A*2402, A*3001	A*2403, A*2404, A*3002, A*3003
B7	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*3503, B*3504, B*3505, B*3506, B*3507, B*3508, B*5101, B*5102, B*5103, B*5104, B*5105, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801	B*1511, B*4201, B*5901
B27	B*1401, B*1402, B*1509, B*2702, B*2703, B*2704, B*2705, B*2706, B*3801, B*3901, B*3902, B*7301	B*2701, B*2707, B*3802, B*3903, B*3904, B*3905, B*4801, B*4802, B*1510, B*1518, B*1503
B44	B*1801, B*1802, B*3701, B*4402, B*4403, B*4404, B*4001, B*4002, B*4006	B*4101, B*4501, B*4701, B*4901, B*5001
B58	B*5701, B*5702, B*5801, B*5802, B*1516, B*1517	
B62	B*1501, B*1502, B*1513, B*3201	B*1301, B*1302, B*1504, B*1505, B*1506, B*1507, B*1515, B*1520, B*1521, B*1512, B*1514, B*1510

- a. Verified alleles includes alleles whose specificity has been determined by pool sequencing analysis, peptide binding assays, or by analysis of the sequences of CTL epitopes.
- b. Predicted alleles are alleles whose specificity is predicted on the basis of B and F pocket structure to overlap with the supertype specificity.

Table VIIA
Map 2 A01 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	Λ*0101	SEQ ID NO.
ASEYLQLVF	154	9		1
ASSFSTINY	68	10	0.1700	2
DLVQENNYLEY	249	10		3
ELSMILEYF	224	8		4
ELVIFFLILKY	115	10		5
ESVLRNQDF	137	10		6
ESVLRNQDFF	137	11		7
EVFEGRIEDSVF	229	11		8
EVVPISHLY	168	9	0.0028	9
FSTTINYTLW	71	10		10
GSDPACYEFLW	263	9		11
HISPOGASSF	63	9		12
ILVTCGLGSY	177	10		13
ISRKMVIELVIF	109	11		14
KIGGEPHISY	292	10		15
KMVELVIF	112	8		16
LLMQDLVQENY	245	11		17
LMQDLVQENY	246	10		18
LVHFLILKY	116	9	0.0450	19
LVQENYLEY	250	9		20
LVTICGLGSY	178	9		21
PVFSKASEY	148	10		22
QVPGSDPACY	260	10		23
RMPFDLISEF	96	10		24
SSFSTINY	69	9		25
STTINYTLW	72	9	0.0430	26
SVLRNCQDF	138	9		27
SVLRNCQDFF	138	10		28
TTINYTLW	73	8		29
VIFSKASEY	149	9		30
VLRNCQDF	139	8		31
VLRNCQDFF	139	9		32
VTCLGLSY	179	8		33
WVEVVPISHLY	166	11	0.2000	34
WVPSHLY	169	8		35
YILVTCGLGSY	176	11		36

Table VII B
Mage 3 A01 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASSLIPITMNY	68	10	2.6000	38
ASSSLQLVF	154	9	0.1100	39
ATCLGLSY	179	8		40
ELSVLEVF	224	8		41
ELVHFLLKLY	115	10		42
EMLGSVVGNNW	134	10		43
EVDPIGHLY	168	9		44
EVDPIGHLYIF	168	11		45
FVQENYLEV	250	9		46
GSDPACYEF	263	9		47
GSDPACYEFFLW	263	11		48
GSVVGNWQY	137	9	0.0500	49
GSVVGNWQYF	137	10		50
GSVVGNWQYFF	137	11		51
IISYPPLIIEW	298	10		52
ISGGPHISY	293	9	0.0370	53
ISYPPLIIEW	299	9		54
KISGGPHISY	292	10	0.0011	55
KVAELVIF	112	8		56
LLTOHQFVQENY	245	11		57
LMEVDPIGHLY	166	11	7.5000	58
LSREKVAELVIF	109	11		59
LTCQHIVQENY	246	10	0.2600	60
LVHFLLLKLY	116	9		61
MLGSVVGNNW	135	9		62
MLGSVVGNNWQY	135	11		63
PIGHLYIF	171	8		64
PSTFPDLESIF	95	11		65
PITTMNYPW	72	9		66
QVRPGSDRACY	260	10		67
SUPTTMNY	70	8		68
SUPTTMNYPLW	70	11	0.0550	69
SSLIPTTMNY	69	9		70
SSSSLQLVF	155	8		71
STFPDLESIF	96	10		72
SVVGNWQY	138	8		73
SVVGNWQYF	138	9		74
SVVGNWQYFF	138	10		75
TMNYPPLWSQSY	74	11	0.0830	76
TIMNYPPLW	73	8		77
VVGNWQYF	139	8		78
VVGNWQYFF	139	9		79
YIFATCLGLSY	176	11		80

Table VII.Δ
Map 2 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
AASRKMV	107	8						81
AASRKMVEL	107	10	0.0001					82
AASRKMVELV	107	11						83
AIALEGDCA	207	10	0.0023					84
AISRKMVEL	108	9	0.0003					85
AISRKMVELV	108	10	0.0001					86
ALGLVGAQA	22	9	0.0030					87
ALGLVGAQAV	22	11						88
ALIETSYVV	277	8						89
ALIETSYVKV	277	10	0.0100	0.0059	0.0800	0.0019		90
ALIETSYVKVL	277	11						91
AQAPATEEQQT	28	11						92
ATEEQQTA	32	8						93
CAPEEKIWEEL	215	11						94
CLGLSYDGL	181	9	0.0004					95
CLGLSYDGLL	181	10	0.0001					96
CQDFFPVI	143	8						97
DLESEFOQA	100	8						98
DLESEFOQAA	100	9	0.0001					99
DLESEFOQAAI	100	10	0.0001					100
DLVQENYL	249	8						101
EALGLYGA	21	8						102
EALGLYGAQAA	21	10	0.0001					103
EARGEAQL	17	9	0.0001					104
EARGEALGLV	17	10	0.0001					105
ELVIELLL	115	8						106
EQQTASSST	35	10						107
EQQTASSSSL	35	11						108
ETSYVKVLYL	280	8						109
ETSYVKVLIHT	280	11						110
EVFEGREDSV	229	10	0.0003					111
EVLGEVPA	47	9	0.0001					112
EVTLGIEVPA	47	10	0.0001					113
EVVEVVPPI	165	8						114
EVVVEVVPPIHL	165	11						115
EVVPISHL	168	8						116
EVVPISHLYI	168	10						117
EVVPISHLYIL	168	11						118
FALIPRKL	239	8						119
FAHPRKLIM	239	9						120
FLLKYRA	119	8						121
FLWGPRAL	271	8						122
FLWGPRALI	271	9						123
FLWGPRALIT	271	11						124
FQAAISRKMV	105	9						125
GASSFSTT	67	8						126
GASSFSTT	67	9						127
GIEVVVEV	163	8	0.0001					128
GIEVVVEV	163	10	0.0002					129

Table VIII A
Mage 2 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*0802	SEQ ID NO.
GLEARGEA	15	8	9	9	9	9	9	131
GLEARGEALGL	15	11	—	—	—	—	—	132
GLLGDNQV	188	8	8	8	8	8	8	133
GLLGDNQVM	188	9	9	9	9	9	9	134
GLLIVIA	200	8	8	8	8	8	8	135
GLLIVVLAI	200	9	9	9	9	9	9	136
GLLIVVLAI	200	10	10	10	10	10	10	137
GLSYDGIL	183	8	8	8	8	8	8	138
GLVGAQATA	24	9	9	9	9	9	9	139
GLVGAQAPAT	24	10	10	10	10	10	10	140
IHSYPLHIERA	298	11	11	11	11	11	11	141
HLYLVTCIL	174	9	9	9	9	9	9	142
HLYLVTCILQL	174	11	11	11	11	11	11	143
HTLKIGGEPHII	289	11	11	11	11	11	11	144
IAIEGDCA	209	8	8	8	8	8	8	145
IAIEGDCA	208	9	9	9	9	9	9	146
IVLAIIA	203	8	8	8	8	8	8	147
IVLAIIAI	203	9	9	9	9	9	9	148
IVLAIIAI	204	8	8	8	8	8	8	149
IVLAIIAI	204	9	9	9	9	9	9	150
IUTCLGI	177	8	8	8	8	8	8	151
KAEMLESV	132	8	8	8	8	8	8	152
KAEMLESVL	132	9	9	9	9	9	9	153
KASEYLQL	153	8	8	8	8	8	8	154
KASEYLQLV	153	9	9	9	9	9	9	155
KIGGEPHI	292	8	8	8	8	8	8	156
KIWEELSM	220	8	8	8	8	8	8	157
KIWEELSMI	220	9	9	9	9	9	9	158
KIWEELSMLEV	220	9	9	9	9	9	9	159
KLLMDQLY	244	8	8	8	8	8	8	160
KMVELVIFL	112	9	9	9	9	9	9	161
KMVELVIFL	112	10	10	10	10	10	10	162
KMVELVIFLL	112	11	11	11	11	11	11	163
KTGLLIV	198	8	8	8	8	8	8	164
KTGLLIV	198	9	9	9	9	9	9	165
KTGLLIVV	198	10	10	10	10	10	10	166
KTGLLIVVLA	198	11	11	11	11	11	11	167
KVLIJHTLKI	285	9	9	9	9	9	9	168
LAIIAIEGDCA	206	11	11	11	11	11	11	169
LJETSYVKV	278	9	9	9	9	9	9	170
LJETSYVKVL	278	10	10	10	10	10	10	171
LIVLAI	202	8	8	8	8	8	8	172
LIVLAI	202	9	9	9	9	9	9	173
LIVLAI	202	10	10	10	10	10	10	174
LLGDNQVM	189	8	8	8	8	8	8	175
LLGDNQVNPKT	189	11	11	11	11	11	11	176
LLIVLAI	201	8	8	8	8	8	8	177
LLIVLAI	201	9	9	9	9	9	9	178
LLIVLAI	201	10	10	10	10	10	10	179
LLIVLAI	201	10	10	10	10	10	10	180

Table VIIIΔ
Map 2 Δ02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	Λ*0201	Λ*0202	Λ*0203	Λ*0206	Λ*6802	SEQ ID NO.
LLIVLALAI	201	11						181
LLKYRAREPV	121	10	0.0001					182
LLKYRAREPVT	121	11						183
LLKYRAREPVG	120	11						184
LMDQLYQENYL	246	11						185
LQLVFGIEV	158	9						186
LQLVFGIEVV	158	10		0.0001				187
LVEVILGEV	45	9						188
LVEVILGEVTA	45	11						189
LVEVILGEVTA	160	8						190
LVFGIEVV	160	10						191
LVFGIEVV	160	11						192
LVFGIEVV	160	8						193
LVGAQAPAT	25	9						194
LVHFLLLKRYRA	116	11						195
MQDLYQENYL	247	10						196
MVFLVTHFL	113	8						197
MVELVHFL	113	9						198
MVELVHFLL	113	10						199
NQEEEGPRM	89	9						200
NQVAMPKIGL	193	9						201
NQVMPKIGL	193	10						202
NQVMPKIGLU	193	11						203
PATEEQQT	31	8						204
PATEEQQTΛ	31	9						205
PISIYL	171	8						206
PISHLYILV	171	9						207
PISHLYILV	171	10						208
PQGASSFST	65	9						209
PQGASSFSTT	65	10						210
PQGASSFSTT	65	11						211
PVIESKASEYL	148	11						212
PVTKAEML	129	8						213
PVTKAEMLSV	129	11						214
QAASIRSKM	106	8						215
QAASIRKAV	106	9						216
QAASIRKAVEL	106	11						217
QAPATEEQQT	29	10						218
QAPATEEQQTΛ	29	11						219
QQTASSST	36	8						220
QQTASSSTL	36	10						221
QQTASSSTLV	36	11						222
QTASSST	37	8						223
QTASSSTL	37	9						224
QTASSSTLV	37	10						225
QVMPKTGL	194	8						226
QVMPKTGL	194	9						227
QVMPKTGL	194	10						228
QVMPKTGL	194	10						229
QVMPKTGL	194	8						230

Table VIIA
Mage 2 A02 Supermotif with Binding Data

Table VIII A
Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
QVMPKTGGLI	194	10	0.0002					231
QVPGSDPA	194	11						232
RALIETSYV	260	8						233
RALIETSYVK	276	9	0.0017					234
RAREPVTKA	276	11						235
RAREPVTKAEM	125	9						236
RQVPGSDPA	259	9						237
SQICKPREGL	7	10						238
STLVIEVTL	43	8						239
STLVIEVTLGEV	43	11	0.0140					240
STTINVTL	72	8						241
SVFAIIPRKLL	237	9	0.0046					242
SVFAIIPRKLLM	237	10	0.0011					243
SVFAIIPRKLLM	237	11						244
TASSSSSTL	38	8						245
TASSSSSTLV	38	9	0.0001					246
TASSSSSTLVEV	38	11						247
TLGEVTVAA	49	8						248
TLKIGGGFIH	290	10	0.0001					249
TLVVEVTLGEV	44	10	0.0250					250
VIFSKASIEYL	149	10	0.0014					251
VLIHTITLKI	286	8						252
VLRNCQDFFPV	139	11						253
VMPKTGGLI	195	8						254
VMPKTGGLI	195	9	0.0010					255
VMPKTGGLIIV	195	10	0.0009					256
VQENNYLEYRQV	251	11						257
VTCLGLSYDGL	179	11						258
VTKAEMLIESV	130	10						259
VTKAEMLIESV	130	11						260
VTLGEVPA	48	8						261
VTLGEVPA	48	9	0.0045					262
VVPISULYI	166	10	0.0002					263
VVPISULYI	169	9	0.0002					264
VVPISULYI	169	10	0.0002					265
VILVTCGL	176	9						266
YLQLVFGI	157	8						267
YLQLVFGIEV	157	10						268
YLQLVFGIEV	157	11						269
YVKVLIIHIT	283	8						270
YVKVLIIHIT	283	9	0.0001					271
YVKVLIIHIT	283	9	0.0001					272
YVKVLIIHIT	283	10	0.3700					273
YVKVLIIHIT	283	11						274

Map 3 A02 Supermotif with Binding Data

Table VIII B
Map 3 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*0209	SEQ ID NO.
AALSRKVVA	107	8						276
AALSRKVAELV	107	10	0.0007					277
AALSRKVAELV	107	11						278
AASSSSTLV	38	8						279
AASSSSTLV	38	9	0.0001					280
AASSSSTLVEV	38	11						281
AIARGGDCA	207	10	0.0002					282
ALGLYGAQA	22	9	0.0030					283
ALGLYGAQAA	22	11						284
ALSRKVAEL	108	9	0.0050					285
ALSRKVAELV	108	10	0.0001					286
ALVETSVV	277	8						287
ALVETSYVKV	277	10	0.0024					288
ALVETSYVKV	277	11						289
AQAPATEEQEAA	28	11						290
ATCLGLSYDGL	179	11						291
ATEEQEAA	32	8						292
CATEEKIWEEL	215	11						293
CLGLSYDGL	181	9	0.0004					294
CLGLSYDGL	181	10	0.0001					295
DLESEFQAA	100	8						296
DLESEFQAA	100	9	0.0001					297
DLESEFQAA	100	10	0.0001					298
EAASSST	37	8						299
EAASSSSL	37	9	0.0001					300
EAASSSSL	37	10	0.0001					301
EALGLVGA	21	8						302
EALGLVGAQA	21	10	0.0001					303
FARGEALGL	17	9	0.0001					304
FARGEALGL	17	10	0.0001					305
ELMEVDPI	165	8						306
ELMEVDPI	165	11	0.0260					307
ELVHFLL	115	8						308
EQEAAASSST	35	10						309
EQEAAASSSTL	35	11						310
EITSYVKV	280	8						311
EITSYVKVLIHM	280	11						312
EVDPIGHL	168	8						313
EVDPIGHLYI	168	10	0.0002					314
EVFEGREDSI	229	10	0.0001					315
EVFEGREDSIL	229	11	0.0001					316
EVTLGIVPAA	47	9	0.0001					317
FLLKYRA	119	8						318
FLWGPRAL	271	8						319
FLWGPRALV	271	9						320
FQAAALSRKV	105	11						321
FQAAALSRKV	105	9						322
GASSLPTT	67	10						323
GASSLPTT	67	8						324
						0.0043	1.1000	325
						0.9100	0.0500	

Table VIII B
Mage 3 Δ02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	Λ*0201	Λ*0202	Λ*0203	Λ*0206	Λ*6802	SEQ ID NO.
GASSLPTTM	67	9	0.0001					326
GIELMEVIDP	163	10	0.0002					327
GLEARGEA	15	8						328
GLEARGEAL	15	9	0.0001					329
GLEARGEALGL	15	11						330
GLLGDNQI	188	8						331
GLLGDNQIM	188	9						332
GLLIIVLVA	200	8						333
GLLIIVLAI	200	9						334
GLLIIVLAI	200	10						335
GLLIIVLAIK	200	11						336
GLSYDGLL	183	8						337
GLVGAQAYA	24	9	0.0003					338
GLVGAQAPAT	24	10	0.0004					339
HISYPPLHFWV	298	11						340
IILYIFATCL	174	9	0.0003					341
HLYIFATCLGL	174	11						342
HMVKISGGPHI	289	11						343
IAREGIDCA	209	8	0.0001					344
IAREGIDCA	208	9						345
IVLVLIA	203	8						346
ILGDPKKL	238	8						347
ILGDPKKLL	238	9	0.0001					348
ILGDPKKLLT	238	10	0.0001					349
IMPKGALL	195	8						350
IMPKGALLI	195	9	0.0064					351
IMPKGALLII	195	10	0.0015					352
IMPKGALLIV	195	11	0.0130					353
KAEMILGSV	132	8						354
KAEMLGSVV	132	9	0.0001					355
KAGLILIV	198	8						356
KAGLILIVL	198	9	0.0002					357
KAGLILIVLV	198	10	0.0002					358
KAGLILIVLAI	198	11						359
KASSSLQL	153	8						360
KASSSLQLV	153	9	0.0005					361
KISGGPHI	292	8						362
KIWEELSV	220	8						363
KIWEELSVLEV	220	9						364
KLTTQIEV	244	8						365
KVAELVHFL	112	9	0.0550					366
KVAELVHFLL	112	10	0.0120					367
KVAELVHFLL	112	11						368
KVLIHHMVKI	285	9						369
LAIAREGIDCA	206	11						370
LIVLAI	202	8						371
LIVLAI	202	9						372
LLGDNQIM	189	8						373
LLGDNQIMPKA	189	11						374

Table VIII B
Mage 3 A02 Supermotif with Binding Data

SEQ ID NO.	Position	Sequence	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802
376	201	LIVLAI	8	0.0001	0.0001	0.0001	0.0001	0.0001
377	201	LIVLALI	9	0.0001	0.0002	0.0002	0.0002	0.0001
378	201	LIVLVALIAA	10	0.0001	0.0001	0.0001	0.0001	0.0001
379	121	LKRYRAREPV	10	0.0001	0.0001	0.0001	0.0001	0.0001
380	121	LKRYRAREPV	11	0.0001	0.0001	0.0001	0.0001	0.0001
381	120	LKRYRAREPV	11	0.0001	0.0005	0.0005	0.0005	0.0001
382	166	LNEVDIGIL	10	0.0001	0.0002	0.0002	0.0002	0.0001
383	158	LQVFGIEL	9	0.0001	0.0001	0.0001	0.0001	0.0001
384	158	LQVFGIELM	10	0.0001	0.0001	0.0001	0.0001	0.0001
385	246	LTOHVFQENYL	11	0.0001	0.0001	0.0001	0.0001	0.0001
386	278	LVEISYVKV	9	0.0001	0.0002	0.0002	0.0002	0.0001
387	278	LVEISYVKVL	10	0.0001	0.0002	0.0002	0.0002	0.0001
388	45	LVEVTLGEV	9	0.0001	0.0001	0.0001	0.0001	0.0001
389	45	LVEVTLGEVPA	11	0.0001	0.0001	0.0001	0.0001	0.0001
390	160	LVFGIELM	8	0.0001	0.0002	0.0002	0.0002	0.0001
391	160	LVFGIELMEV	10	0.0001	0.0002	0.0002	0.0002	0.0001
392	25	LVGAQAPA	8	0.0001	0.0001	0.0001	0.0001	0.0001
393	25	LVGAQAPAT	9	0.0001	0.0001	0.0001	0.0001	0.0001
394	116	LVIFLLLKYRA	11	0.0001	0.0002	0.0002	0.0002	0.0001
395	290	MVKISGGPHI	10	0.0002	0.0002	0.0002	0.0002	0.0001
396	89	NQEEEGPST	9	0.0001	0.0001	0.0001	0.0001	0.0001
397	193	NQIMPKAGL	9	0.0001	0.0001	0.0001	0.0001	0.0001
398	193	NQIMPKAGLI	10	0.0001	0.0001	0.0001	0.0001	0.0001
399	193	NQIMPKAGLI	11	0.0001	0.0002	0.0002	0.0002	0.0001
400	31	PATEEQQLA	8	0.0001	0.0001	0.0001	0.0001	0.0001
401	31	PATEEQQLAA	9	0.0001	0.0001	0.0001	0.0001	0.0001
402	171	PIGHLYIFIA	9	0.0001	0.0001	0.0001	0.0001	0.0001
403	171	PIGHLYIFAT	10	0.0001	0.0003	0.0003	0.0003	0.0001
404	65	POGASSLPT	9	0.0001	0.0001	0.0001	0.0001	0.0001
405	65	POGASSLPTT	10	0.0001	0.0001	0.0001	0.0001	0.0001
406	65	POGASSLPTTM	11	0.0001	0.0001	0.0001	0.0001	0.0001
407	62	POPOGASSL	10	0.0001	0.0001	0.0001	0.0001	0.0001
408	72	PTTMNVTL	8	0.0001	0.0001	0.0001	0.0001	0.0001
409	148	PVFSKASSL	11	0.0001	0.0001	0.0001	0.0001	0.0001
410	129	PVTKAEML	8	0.0001	0.0001	0.0001	0.0001	0.0001
411	129	PVTKAEMLGSV	11	0.0001	0.0001	0.0001	0.0001	0.0001
412	106	QAAALSRKV	8	0.0001	0.0001	0.0001	0.0001	0.0001
413	106	QAAALSRKVAA	9	0.0001	0.0001	0.0001	0.0001	0.0001
414	106	QAAALSRKVAAEL	11	0.0001	0.0001	0.0001	0.0001	0.0001
415	129	QAAATEEQEA	10	0.0001	0.0001	0.0001	0.0001	0.0001
416	159	QIMPKAGL	8	0.0001	0.0001	0.0001	0.0001	0.0001
417	194	QIMPKAGLL	9	0.0001	0.0001	0.0001	0.0001	0.0001
418	194	QIMPKAGLLI	10	0.0001	0.0006	0.0006	0.0006	0.0001
419	194	QIMPKAGLLII	11	0.0001	0.0001	0.0001	0.0001	0.0001
420	159	QLVFGIEL	8	0.0001	0.0001	0.0001	0.0001	0.0001
421	159	QLVFGIELM	9	0.0001	0.0001	0.0001	0.0001	0.0001
422	159	QLVFGIELMEV	11	0.0001	0.3400	0.3400	0.3400	0.0001
423	260	QVPGSDDA	8	0.0001	0.0001	0.0001	0.0001	0.0001
424	276	RALVETSY	9	0.0001	0.0001	0.0001	0.0001	0.0001

Table VIII B
Maple 3 A02 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0201	A*0202	A*0203	A*0206	A*6802	SEQ ID NO.
RALVETSYKV	276	11						426
RAREPVTKAEM	125	9						427
RAREPVTKAEM	125	11						428
RQVGSDPA	259	9						429
SILGDPKKL	237	9	0.0001					430
SILGDPKKL	237	10	0.0002					431
SILGDPKKLT	237	11						432
SUFTTMNYPM	70	10	0.0035					433
SQLVFGI	157	8						434
SQLVFGIEL	157	10	0.0049					435
SQLVFGIELM	157	11						436
SQICKPEEGL	7	10						437
STLVEVTL	43	8						438
STLVEVTLGEV	43	11	0.0140					439
TLGEVPA	49	8						440
TLVEVTLGEV	44	10	0.0250					441
TQIFVQENYL	247	10						442
VAELVIIFL	113	8						443
VAELVIIFL	113	9	0.0001					444
VAELVHFLL	113	10	0.0009					445
VIFSKASSSL	149	10	0.0001					446
VLIHMVKI	286	8						447
VQENYLEYRQV	251	11						448
VTKAEMLGSVV	130	10	0.0002					449
VTKAEMLGSVV	130	11						450
VTLGEPVPA	48	8						451
VTLGEPVPA	48	9	0.0045					452
VVGNNWQYFFPv	139	11						453
WQYFFPv	143	8						454
YIFATCLGL	176	9	0.0180					455
YVKVLHIM	283	8						456
YVKVLHIMV	283	9	0.0001					457
YVKVLHIMVKI	283	11						458

Table IX^A
Map 2 A03 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
AIEGDCAPEEK	210	11	0.0009	0.0007	0.0200	0.0003	0.0280	459
AJETSYVK	277	9	0.0810	0.1900				460
DLYQDENYLEYR	249	11	0.0047	0.0018				461
DSVFAIPR	236	8	-0.0004	0.0005				462
DSVFAHPRK	236	9	0.0021	0.0025	0.0006	0.0190	0.0460	463
ELSMLEVFEGR	224	11	0.0016	0.0008				464
ELVHFLLK	115	9	0.0045	0.0111				465
ELVHFLLKYR	115	11	0.0011	0.0031				466
EMLESTYLR	134	8	-0.0009	-0.0003				467
ESEFOQAISR	102	10	0.0002	0.0002				468
ESEFOQAASRK	102	11	0.0010	0.0004				469
FLLKYRAR	119	9						470
FSTTINYNTLWR	71	11	0.0110	0.0170	0.0700	0.0074	0.0490	471
GLLGDNQVMKP	188	11	0.0780	0.0047	-0.0006	-0.0013	-0.0001	472
GSSNQEEGPR	86	11	-0.0002	-0.0002	0.0018			473
HISYPYLHER	298	10	0.0074	0.0018				474
ISYPLPLIER	299	9	0.0340	0.0280	0.7700	0.8100	0.0990	475
KAEMLVEYLR	132	10	0.0002	0.0009	0.0047		0.0004	476
KVLHHHTLK	285	8	0.0053	0.0100				477
LIELTSYVK	278	8	-0.0004	0.0027				478
LLGDNQVMKP	189	10	0.0093	0.0014				479
LLKYRAR	120	8	-0.0009	-0.0004				480
LSMLEVEFGR	225	10	-0.0004	0.0001				481
LVHFLLLK	116	8	0.0290	0.1500	0.0007	-0.0009	0.0200	482
LVHFLLKYR	116	10	0.0260	0.0022				483
LVQENTYLEYR	250	10	0.0027	0.0089				484
MLEVFGGR	227	8	-0.0009	-0.0004				485
MVELYHHLKK	113	11	0.0200	0.0120	0.0038	0.0056	0.0020	486
PACYEFLWGR	266	11	-0.0009	-0.0002				487
PLFQRQSICK	2	10	0.0003	0.0002				488
PLHERALR	303	8	-0.0009	-0.0004				489
RALIETSYVK	276	10	0.0200	0.0750	0.0064	0.0003	0.0026	490
RAREFVTK	125	8	-0.0009	-0.0003				491
SMLEVFGGR	226	9	0.0020	0.0220	0.4900	3.2000	0.0044	492
SSNOQEFGPR	87	10	0.0002	0.0002				493
STTINYNTLWR	72	10	0.0014	0.0910				494
SVFHFRK	237	8	0.1410	0.0810	0.0130	0.0010	0.0440	495
TINYNTLWR	74	8	0.0140	0.0550	0.0250	0.0370	0.3800	496
TTINYNTLWR	73	9	0.0890	1.1000	0.0005	-0.0009	0.0360	497
YVKVLLHHTLK	283	10	0.0033	0.0160				498

Table IX.B
Maple 3 A03 Supermotif with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	A*1101	A*3101	A*3301	A*6801	SEQ ID NO.
ALVETSYVK	277	9	0.0270	0.1700	0.0009	0.0004	0.0022	499
DSILGDPK	236	8	-0.0004	-0.0003	-0.0003	-0.0003	-0.0003	500
DSILGDPKK	236	9	-0.0003	-0.0002	-0.0002	-0.0002	-0.0002	501
ELSVLEVTEGR	224	11	-0.0009	0.0023	0.0023	0.0023	0.0023	502
ELVIFLLK	115	9	0.0045	0.0011	0.0011	0.0011	0.0011	503
ELVIFLLKVR	115	11	0.0011	0.0031	0.0031	0.0031	0.0031	504
ESEFOAALSRSR	102	10	0.0002	0.0002	0.0002	0.0002	0.0002	505
ESEFOAALSRSR	102	11	0.0002	0.0004	0.0004	0.0004	0.0004	506
FLLKYRAR	119	9	0.0009	0.0012	0.0012	0.0012	0.0012	507
FYQENYLEYR	250	10	0.1300	0.0570	-0.0006	-0.0013	-0.0001	508
GLLGDNQIMPK	188	11	0.0069	0.0011	0.0011	0.0011	0.0011	509
IIVLAIAR	203	9	0.0053	0.0037	0.0037	0.0037	0.0037	510
IVLAIAR	204	8	0.0580	0.0190	0.0190	0.0190	0.0190	511
KVLIHIMVK	285	8	0.0280	0.0021	0.0021	0.0021	0.0021	512
LIVLAIAR	202	10	0.0200	0.0110	0.0110	0.0110	0.0110	513
LLGIDNQIMPK	189	10	0.0021	0.0056	0.0056	0.0056	0.0056	514
LIVLAIAR	201	11	-0.0009	-0.0004	-0.0004	-0.0004	-0.0004	515
LLLKYRAR	120	8	-0.0006	0.0030	0.0030	0.0030	0.0030	516
LSVLEVFEGR	225	10	-0.0004	0.0014	0.0014	0.0014	0.0014	517
LVETSYVK	278	8	0.0290	0.1500	0.0007	-0.0009	0.0200	518
LVIHLKLK	116	8	0.0260	0.0022	0.0022	0.0022	0.0022	519
LVIHLKLK	116	10	-0.0009	-0.0002	-0.0002	-0.0002	-0.0002	520
PACYEFIWGR	266	11	0.0003	0.0002	0.0002	0.0002	0.0002	521
PLEQRSQICK	2	10	-0.0003	0.0002	0.0002	0.0002	0.0002	522
PLIHEWVLR	303	8	-0.0009	0.0003	0.0003	0.0003	0.0003	523
RALVETSYVK	276	10	0.0190	0.1100	0.0034	0.0003	0.0004	524
RAREPVTK	125	8	-0.0009	-0.0003	-0.0003	-0.0003	-0.0003	525
SILGDPKK	237	8	-0.0009	0.0012	0.0012	0.0012	0.0012	526
SVLEVFEGR	226	9	0.0003	0.1400	0.1700	0.1600	0.0860	527
VAVLVIFLLK	113	11	-0.0002	0.0011	0.0011	0.0011	0.0011	528
VLEVFEGR	227	8	0.0016	0.0005	0.0005	0.0005	0.0005	529
YYKVVLHIMVK	283	10	0.0020	0.0061	0.0061	0.0061	0.0061	530

Table XA
Mage 2 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
AISRKMKVEL	108	9		531
ALIFTSYVKVL	277	11		532
CLGLSYDGLL	181	9		533
CLGLSYDGLL	181	10		534
CYEFLVGPRAL	268	11	0.0004	535
DLESEFQAAI	100	10		516
DLVQENYL	249	8		537
DLVQENYL	249	10		538
EFLWGPRAL	270	9	0.0006	539
EFLWGPRAL	270	10	0.0097	540
EFLWGPRAL	104	10	0.0002	541
EFOAAISRKM				542
ELSMIJEVF	224	8		543
ELVIFLLL	115	8		544
ELVIFLLL	115	10		545
ETSYVKVL	280	8		546
EVFEGRIEDSVF	229	11		547
EVVEVVPPI	165	8		548
EVVEVVPPI	165	11		549
EVVPISHL	168	8		550
EVVPISHL	168	9		551
EVVPISHL	168	10		552
EVVPISHL	168	11		553
EVVPISHL	168	11	3.5000	554
EVVPISHL	168	11		555
EVVPISHL	168	11		556
EYLOLYFGI	156	9		557
FLWGPRAL	271	8		558
FLWGPRAL	271	9		559
GIEVVVEVVPPI	163	10		560
GLEARGEAL	15	9		561
GLEARGEAL	15	9		562
GLLGIDINQWM	188	9		563
GLLIVLVAI	200	9		564
GLLIVLVAI	200	10		565
GLSYDGLL	183	8		566
HLYLVTCL	174	9		567
HLYLVTCL	174	11		568
HTLKIGGEPHI	289	9		569
IFSKASEY	150	8		570
IFSKASEY	150	9	0.0230	571
IFSKASEY	150	9	0.0950	572
IVLAIAAI	203	11	0.0007	573
IVLAIAAI	203	9	0.0170	574
ILVTCLGL	177	8		575
ILVTCLGL	177	10		576
IVLAIAAI	204	8		577
IWEELSML	221	8		578
IWEELSML	221	11		579
KIGGEPHI	292	8		580
KIGGEPHSY	292	10		
KIWEELSM	220	8		
KIWEELSM	220	9		
KMVELVIF	112	8		
KMVELVIF	112	9		

Table XA
Mage 2_A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
KMVELVIFLL	112	10		581
KMVELVIFLL	112	11		582
KTGLLIVL	198	9		583
KIGLLIVLAI	198	11		584
KVLJHITKIL	285	9		585
LIFISYYVKVL	278	10		586
LIVVLAILI	202	8		587
LIVVLAILI	202	10		588
LLGDQNQVM	189	8		589
LJIVVLALI	201	8		590
LJIVVLALI	201	9		591
LJIVVLALI	201	11		592
LJMDPLVQENY	245	11		593
LMDPLVQENY	246	10		594
LMDPLVQENYL	246	11		595
LVHFLLKLV	116	9		596
LVQENYLEY	250	9		597
LVTICLGLSV	178	9		598
LWGPRALI	272	8		599
LYLIVTCL	175	8		600
LYLIVTCLGL	175	10		601
MFPDPLESFF	97	9		602
MVELVIFL	113	8		603
MVELVIFL	113	9		604
MVELVIFLL	113	10		605
PISHLVYL	171	8		606
PVIFSKASEY	148	10		607
PVIFSKASEYL	148	11		608
PVTKAEML	129	8		609
QIASSSETL	37	9		610
QVMPIKTGL	194	8		611
QVMPIKTGLL	194	9		612
QVMPIKTGLL	194	10		613
QVPGSDPACY	260	11		614
RNFDPDLESFF	96	10		615
SFSITINY	194	8		616
SFSITINYTL	70	8		617
SFSITINYTLW	70	10		618
STLVEVIL	43	8		619
STTINNYL	72	8		620
STTINNYLW	72	9		621
SVFAIIPRKL	237	9		622
SVFAIIPRKL	237	10		623
SVFAIIPRKLMM	237	11		624
SVLRNQDF	138	9		625
SVLRNQDF	138	10		626
SYPLIJERAL	300	10		627
SYVKVLIHTL	282	10		628
TKIGGEPHII	290	10		629
			0.0003	0.01600

Table XA
Map 2. A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
TTINYTLW	73	8		631
VFAIIPRKI	238	8	0.0005	632
VFAIIPRKLL	238	9	0.0006	633
VFAIIPRKLLM	238	10		634
VFIGREDGSVF	230	10	0.0004	635
VIFSKASEY	149	9		636
VIFSKASEYL	149	10		637
VLIIHITLKI	286	8		638
VLRNQCQDF	139	8		639
VLRNQDFF	139	9		640
VMPKIGLL	195	8	-0.0004	641
VMPKIGLLI	195	9	0.2300	642
VMPKIGLLII	195	10	0.0580	643
VTCGLGSLY	179	8		644
VTCGLGSLYDGL	179	11		645
VTKAEMLISVL	130	11		646
VVEVVPISHIL	166	10		647
VVEVVPISHILY	166	11		648
VVPISHLY	169	8		649
VVPISHLYI	169	9		650
VVPISHLYIL	169	10		651
YILVTCGLGL	176	9		652
YILVTCGLGSLY	176	11		653
YLQLVFGH	157	8		654
YVKVLIHITL	283	9		655
YVKVLIHITLKI	283	11		656

Table X B
Mage 3 A24 Supernotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
ALSRKVVAEL	108	9		657
ALVETSYVKVL	277	11		658
AT'CLGLSY	179	8		659
ATCLGLSYDGL	179	11		660
CLGLSYDGL	181	9		661
CLGLSYDGL	181	10		662
CYFLWGPRL	268	11	0.0004	663
DLESEFQAAAL	100	10		664
EFLWGPKAL	270	9		665
ELMEVDPI	165	8	0.0006	666
ELMEVDPIGHIL	165	11		667
ELSVLLEVF	224	8		668
ELVIFILL	115	8		669
ELVIFILLKY	115	10		670
EMLGSVVGNW	134	10	0.0017	671
ETSYVKVL	280	8		672
ETSYVKVLHIM	168	11		673
EVDPIGIL	168	8		674
EVDPIGHLY	168	9		675
EVDPIGHLYI	168	10		676
EVDPIGHLYIF	168	11		677
EVFEREDSI	229	10		678
EVFLGREDSI	229	11		679
FLWGPKAL	271	8		680
FYQENYLEY	250	9		681
GIELMEVDPI	163	10		682
GLEARGLIAL	15	9		683
GLEARGLIAL	15	11		684
GLLGDNQI	188	8		685
GLLGDNQIM	188	9		686
GLLIIVLAI	200	9		687
GLLIIVLAI	200	10		688
GLSYTDGLL	183	8		689
HIVQENYL	249	8		690
HIVQENYLEY	249	10		691
IHSYPPHLIEW	298	10		692
HL YIFATCL	174	9		693
IILYIFATCLGL	174	11		694
JMVKISGGPHI	289	11		695
IFATCLGL	177	8		696
IFATCLGLSY	177	10	0.0120	697
IFSKASSSL	150	9	0.0160	698
IFSKASSSLQL	150	11	0.0010	699
ILGDPKKL	238	8		700
ILGDPKKL	238	9		701
IMPKAGLL	195	8		702
IMPKAGLL	195	9	0.4200	703
IMPKAGLLII	195	10	0.0500	704
IWEELSVLF	221	8	-0.0004	705
IWEELSVLF	221	11	0.0260	706

Table X.B
Map 3 A24 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
KISGGRII	292	8		707
KISCGPHISY	292	10		708
KIWEELSVL	220	9		709
KVAELVIF	112	8		710
KVAELVIFL	112	9		711
KVAELVIFLL	112	10		712
KVAELVIFLLL	112	11		713
KVLHIMVKI	285	9		714
LIVLAH	202	8		715
LLGDNQNM	189	8		716
LLIVLVAI	201	8		717
LLTQHIFVQENY	201	9		718
LMEVDPIGIL	245	11		719
LMEVDPIGHLY	166	10		720
LTOHQHIVQENY	166	11		721
LTQHIVQENYL	246	10		722
LVIETSYVKVL	278	10		723
LVFGHELM	160	8		724
LVIFLLKKY	116	9		725
LYIFATCICL	175	8		726
LYIFATCICL	175	10		727
MLGSVYVGNW	135	9	0.0480	728
MLGSVYVGNWQY	135	11		729
MVKISGGRII	290	10		730
NWQYFFFVFI	142	9	0.5300	731
NWQYFFFVIF	142	10	0.0170	732
NYPLWSQSY	76	9	0.0270	733
PIGLYLIF	171	8		734
PTIMNNYPL	72	8		735
PTTMNNYPLW	72	9		736
PVIFSKASSL	148	11		737
PVTKAIEML	129	8		738
QIMPKAGL	194	8		739
QIMPKAGLL	194	9		740
QIMPKAGLLI	194	10		741
QIMPKAGLLII	194	11		742
QLVFGIEL	159	8		743
QLVFGIELM	159	9		744
QVPGSDPACY	260	10		745
QYFFFVIF	144	8		746
SILGDYIKKL	237	9	0.1200	747
SILGDYIKKL	237	10		748
SLPTTMNMY	70	8		749
SLPTTMNYP	70	10		750
SLPTTMNYPW	70	11		751
SLQLVFGIEL	157	8		752
SLQLVFGIELM	157	10		753
STFDLSEF	96	11		754
		10		755
				756

Table X B
Map 3 A24 Supernatant Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
STLVEVTL	43	8		757
SVVGNWQY	138	8		758
SVVGNWQYFF	138	9		759
SVVGNWQYFF	138	10		760
SYDGILGIDNQI	185	11	0.0026	761
SYPLIHEW	300	8	0.0420	762
SYPLIHEWVL	300	10	0.5900	763
SYVKVLIIM	282	9		764
TPDLESEFF	97	9	0.0049	765
TMNYPLWSQSY	74	11		766
TTMNYPLW	73	8		767
VFIGREDISI	230	9	-0.0004	768
VFIGREDISI	230	10	-0.0005	769
VIFSKASSSL	149	10		770
VLIIMVVKI	286	8		771
VVGNWQYF	139	8		772
VVGNWQYFF	139	9		773
YIFATCLGL	176	9		774
YIFATCLGLSY	176	11		775
YVKVLIIM	283	8		776
YVKVLIIMVVKI	283	11		777

Table XI A
Mage 2_B07 Supermotif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
APATEEQQIA	30	10	0.0002	778
APEEKIWEEL	216	10	0.0001	779
DPACYEFL	265	8	-0.0002	780
DPACYEFLW	265	9	0.0001	781
EPHSYVPL	296	9	0.1100	782
EPVTKAEM	128	8	0.0010	783
EIVTKAEML	128	9	0.0001	784
FIDLESIEF	98	8	-0.0002	785
FIDLESIEFQAA	98	10	0.0002	786
FIDLESIEFQAA	98	11	-0.0001	787
FPIVIESKAA	147	8	0.0003	788
FPIVIESKAEY	147	11	0.0004	789
GPRALIETSY	274	10	0.0008	790
GPRALIETSYV	274	11	0.1300	791
GPRMFPDL	94	8	0.0063	792
HPRKLIMQDL	241	10	0.0400	793
HPRKLIMQDLV	241	11	0.0042	794
KPEEGLEA	11	8	-0.0002	795
MPKTGILLI	196	8	0.0190	796
MPKTGILLI	196	9	0.0020	797
MPKTGILLIV	196	10	0.0003	798
MPKTGILLIVL	196	11	0.0099	799
PPHSPOQGA	61	8	-0.0002	800
PPHSPOQASSF	61	11	-0.0003	801
PPLHERAL	302	8	0.0026	802
SPPHSPOQGA	60	9	0.0001	803
STQGASSF	64	8	0.0007	804
SPSPPHSPQGA	58	11	0.0006	805
VPGSDPACY	261	9	0.0001	806
VPGSDPACYEF	261	11	-0.0001	807
VPISHLYI	170	8	0.0170	808
VPISHLYL	170	9	0.2500	809
VPISHLYLV	170	10	0.0027	810
YPLHIERA	301	8	-0.0002	811
YPLHIERAL	301	9	0.2700	812

Mage 3 B07 Supermotif Peptides with Binding Data
Table XI B

Sequence	Position	No. of Amino Acids	B*0702	SEQ ID NO.
APATEEQEA	30	9	0.0001	813
APATEEQEAA	30	10	0.0002	814
APIEKIKWEEL	216	10	0.0001	815
DIVACYEFL	265	8	-0.0002	816
DPACYEFW	265	9	0.0001	817
DPIGHLYI	170	8	-0.0002	818
DPIGHLYIF	170	9	0.0001	819
DPIGHLYIAA	170	10	0.0002	820
DPKELLTQHIF	241	10	0.0001	821
DPKKLLTQHIFV	241	11	-0.0004	822
DPQSPQGA	69	9	0.0001	823
EPIVTKAEM	128	8	0.0010	824
EPIVTKAEML	128	9	0.0001	825
FPPDLESEF	98	8	-0.0002	826
FPPDLESEFQAA	98	10	0.0002	827
FPTLDESEFQAA	98	11	-0.0001	828
FPTLDESEFQAA	147	8	0.0003	829
GPHISYTPL	296	9	0.3800	830
GPRALVETSY	274	10	0.0002	831
GPRALVETSYV	274	11	0.1900	832
GPSTEPDL	94	8	-0.0002	833
KPEEGLEA	11	8	-0.0002	834
LPTTMNYPPL	71	9	0.0770	835
LPTTMNYPPLW	71	10	0.0001	836
MPKAGLLI	196	8	0.1300	837
MPKAGLLIJ	196	9	0.0170	838
MPKAGLLIV	196	10	0.0031	839
MPKAGLLIVL	196	11	0.0280	840
PPLHEWVL	302	8	-0.0002	841
PPQSPQGA	61	8	-0.0002	842
PPQSPQASSL	61	11	0.0049	843
SPDPPQSPQGA	58	11	-0.0001	844
SPQGASSL	64	8	0.0081	845
VRGSDPACY	261	9	0.0001	846
VPGSDPACYEF	261	11	-0.0001	847
YPLWSOSV	77	8	-0.0002	848
YPLHEWV	301	8	0.0002	849
YPLHEWVL	301	9	0.0027	850

Table XII A
Mage 2_B27 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AIPRKLLM	240	8	851
AIPRKLLMQDL	240	11	852
AREFVTKAEM	126	10	853
AREPVTKAEML	126	11	854
ARGEALGL	18	8	855
EKIWEELSM	219	9	856
EKIWEELSML	219	10	857
LKIGGEPHI	291	9	858
LKIGGEPHSY	291	11	859
LRNCQDF	140	8	860
LRNCQDFFPVI	140	11	861
PHISYPPL	297	8	862
PHISYQASSF	62	10	863
PKTGLLII	197	8	864
PKTGLLIIVL	197	10	865
PRALIETSY	275	9	866
PRKLIMQDL	242	9	867
PRMFDPDLESEF	95	11	868
QICKPPEEGL	8	9	869
RKLLMQDL	243	8	870
RKMVELVIF	111	9	871
RKMVELVIFLF	111	10	872
SHLYLVITCL	173	11	873
SKASEYQLQ	152	10	874
SKASEYQLQVF	152	9	875
SRKMVELVIF	110	11	876
SRKMVELVIFLF	110	10	877
TKAEMLESVL	131	11	878
VHFLLKY	117	10	879
VKVLIHHTL	284	8	880
VKVLIHHTLK	284	8	881
		10	882

Table XII B
Mage 3 B27 Supernatif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AREPVTKAEM	126	10	883
AREPVTKAEML	126	11	884
ARGEALGL	18	8	885
EKIWEELSVL	219	10	886
GILYFATCL	173	10	887
KLLTQIF	243	8	888
PLISYPPL	297	8	889
PLISYPPLHEW	297	11	890
PKAGLIIH	197	8	891
PKAGLIIVL	197	10	892
PKKLITQIF	242	9	893
PRALVETSY	275	9	894
QUICKPEIGL	8	9	895
QIFVQENY	248	8	896
QIFVQENYL	248	9	897
QIFVQENYLGY	248	11	898
RKVAELVIF	111	9	899
RKVAELVIFL	111	10	900
RKVAELVIFLL	111	11	901
SKASSSSLQL	152	9	902
SKASSSLQLV	152	11	903
SRKVVAELVIF	110	10	904
SRKVVAELVIFL	110	11	905
VIFULLKY	117	8	906
VKISGGPHII	291	9	907
VKISGGPHIISY	291	11	908
VKVLIHIMVKI	284	10	909

Table XIII.A
Map 2 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AASRKMV	107	8	910
AASRKMVEL	107	10	911
AASRKMVELV	107	11	912
ASEYQLV	154	8	913
ASEYQLQVF	154	9	914
ASEYQLVFGI	154	11	915
ASSFSTI	68	8	916
ASSFSTINY	68	10	917
ASSSSTLV	39	8	918
ASSSSTLVEV	39	10	919
CAPFEKIW	215	8	920
CAPFEKIWEEEL	215	11	921
DSVFAIPRKLL	236	10	922
DSVFAIPRKLL	236	11	923
EARGEALGL	17	9	924
EARGEALGV	17	10	925
ESEFOAAL	102	8	926
ESVLRNQCQDF	137	10	927
ESVLRNQCQDFF	137	11	928
EISVVKVL	280	8	929
FAIIPRKLL	239	8	930
FAIIPRKLLM	239	9	931
FSKASEYIL	151	8	932
FSKASEYILQL	151	10	933
FSKASEYILQLV	151	11	934
FSTTINYTL	71	9	935
FSTTINYTLW	71	10	936
GASSFSTI	67	9	937
GASSFSTINY	67	11	938
GSDPACYEF	263	9	939
GSDPACYEFL	263	10	940
GSDPACYEFLW	263	11	941
HSPQGASSF	63	9	942
ITTLKGGEPII	289	11	943
ISHLYLIV	172	8	944
ISHLYLVTCL	172	11	945
ISRKMVEL	109	8	946
ISRKMVELVIF	109	9	947
ISYPPIHERAL	299	11	948
KAEMLESV	132	8	949
KAEMLESVL	132	9	950
KASEYQLQL	153	8	951
KASEYQLQV	153	9	952
KASEYQLVF	153	10	953
KTGLLIV	198	8	954
KTGLLIVL	198	9	955
KTGLLIVLAI	198	11	956
PACYEFLW	266	8	957
QAAISRKM	106	8	958
			959

Table XIII A
Mage 2_B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
QMAISRKMV	106	11	960
QTAASSSSTL	37	9	961
QTAASSSSTLV	37	10	962
RALETSY	276	8	963
RALIEISYV	276	9	964
RALIETSYYV	276	11	965
RAREPVTKAEM	125	11	966
RSQUICKPPEGL	6	11	967
SSFSTTINY	69	9	968
SSFSTTINYL	69	11	969
SSNQEIEGPRM	87	11	970
SSSSTLVEV	40	9	971
SSSSTLVEVTL	40	11	972
SSSTLVEV	41	8	973
SSSTLVEVTL	41	10	974
SSTLVEVTL	42	9	975
SILVEVTL	43	8	976
STLVEVTL	43	11	977
STLVEVTLGEV	43	11	978
STTINYTL	72	8	979
STTINYTLW	72	9	980
TASSSSTL	38	8	981
TASSSSTLV	38	9	982
TASSSSTLVEV	38	11	983
TSYVKVLHHTL	281	11	984
TTINYTLW	73	8	985
VTCLGLSY	179	8	986
VTCLGLSYDGL	179	11	987
VTKAEMLESV	130	10	988
VTKAEMLESVL	130	11	989

Table XIII B
Mage 3 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AALSRKVVAEL	107	10	990
AASSSSSTL	107	11	991
AASSSSSTLV	38	8	992
AASSSSSTLVEV	38	9	993
ASSLITTM	38	11	994
ASSLITTMNYY	68	8	995
ASSSLQLV	68	10	996
ASSSLQLVF	154	8	997
ASSSLQLVFGI	154	9	998
ASSSSTLV	154	11	999
AASSSTLVEV	39	8	1000
AICLGGSYGL	39	10	1001
CAPEEKIW	179	8	1002
CAPEEKIWELL	215	11	1003
DSELGDPKKL	215	8	1004
DSELGDPKKL	236	10	1005
DSELGDPKKL	236	11	1006
EAASSSSSTL	37	9	1007
EAASSSSSTLV	37	10	1008
EARGEALGL	17	9	1009
EARGEALGV	17	10	1010
ESEFOQAL	102	8	1011
ETSYVKVVL	280	8	1012
FTSYVKVLIIM	280	8	1013
FATCLGLSY	178	11	1014
FSKASSSL	151	9	1015
FSKASSSLQL	151	8	1016
FSKASSSLQLV	151	10	1017
GASSLPTTM	67	11	1018
GASSLPTTMNYY	67	9	1019
GSDPACYEEF	263	11	1020
GSDPACYEEFL	263	9	1021
GSDPACYEEFW	263	10	1022
GSVVGNWQY	137	11	1023
GSVVGNWQYFF	137	9	1024
ISGGPHISY	293	9	1025
ISYPPLIEWV	299	8	1026
ISYPPLIEWV	299	10	1027
KAGLLIIVL	198	8	1028
KAGLLIIVL	198	9	1029
KAGLLIIVL	198	11	1030
KAEMLGSV	132	8	1031
KAEMLGSVV	132	9	1032
KAGLLIIV	198	8	1033
KAGLLIIVL	198	9	1034
KAGLLIIVL	198	11	1035
KASSSLQL	153	8	1036
KASSSLQLV	153	9	1037
KASSSLQLVF	153	10	1038
LSRKVVAEL	109	8	1039

Table XIIIIB
Mape 3 B58 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LSRKVVAELV	109	9	1040
LSRKVVAELVIF	109	11	1041
LTOHFVQENY	246	10	1042
LTOHFVQENYL	246	11	1043
PACYEFLW	266	8	1044
FSTFDLSEEF	95	11	1045
WTTMNYPL	72	8	1046
WTTMNYPLW	72	9	1047
QALSRKV	106	8	1048
QALSRKVVAEL	106	11	1049
OSPQGASSL	63	9	1050
RALVETSY	276	8	1051
RALVETSYV	276	9	1052
RALVETSYVKV	276	11	1053
RAREPVTKAEM	125	11	1054
RSQHICKPEEGL	6	11	1055
SLSPTIMNY	69	9	1056
SLSPTIMNYPL	69	11	1057
SSSQLVFGI	156	9	1058
SSSQLVFGIEI	156	11	1059
SSSQLQVF	155	8	1060
SSSQLQVFGL	155	10	1061
SSSSTLVEV	40	9	1062
SSSSTLVEVL	40	11	1063
SSSSTLVEV	41	8	1064
SSSSTLVEVTL	41	10	1065
SSTLVEVTL	42	9	1066
SSTFDLSEEF	96	10	1067
SILVEVTL	43	8	1068
SILVEVTLGEV	43	11	1069
TSYVKVLIIMV	281	10	1070
TSYVKVLIIMV	281	11	1071
WTTMNYPLW	73	8	1072
VAFLVVFL	113	8	1073
VAFLVVFL	113	9	1074
VAFLVVFLLL	113	10	1075
VTKAEMLGSV	130	10	1076
VTKAEMLGSVV	130	11	1077

Table XIV A
Mage 2_B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
AISPKMVELV	108	10	1078
ALIETSYV	277	8	1079
ALIETSYVKV	277	10	1080
CQDFPFV	143	8	1081
CQDFPFVIF	143	9	1082
DLESFQAAI	100	10	1083
DLVQENLEY	249	10	1084
DPACYEFLW	265	9	1085
ELSMLEVF	224	8	1086
ELVIFILLKY	115	10	1087
EIVTKAEM	128	8	1088
EVFGEGEDSV	229	10	1089
EVFGEGEDSVF	229	11	1090
EVVEVVP	165	8	1091
EVVPIHILY	168	9	1092
EVVPIHILYI	168	10	1093
FLWGPRLI	271	9	1094
FPDIESEF	98	8	1095
FPPVFSKASEY	147	11	1096
FQAAISRKM	105	9	1097
FQAAISRKMV	105	10	1098
GHEVVEVV	163	8	1099
GIEVVEVVP	163	10	1100
GLLGDDNQV	188	8	1101
GLLGDDNQVM	188	9	1102
GLLIVLAI	200	9	1103
GLLIVLAI	200	10	1104
GPRALIETSY	274	10	1105
GPRALIETSYV	274	11	1106
HPRKLIMQDLV	241	11	1107
IIVLAI	203	9	1108
ILVTCLGLSY	177	10	1109
IVLAI	204	8	1110
KIGGEPHI	292	8	1111
KIGGEPHSY	292	10	1112
KIWEELSMLEV	220	8	1113
KLLMQLDV	244	8	1114
KMVELVLF	112	8	1115
KVLHHTLKI	285	9	1116
LIELTSYVKV	278	9	1117
LIVLAI	202	8	1118
LIVLAI	202	10	1119
LLGDQNQVM	189	8	1120
LIVLAI	201	8	1121
LIVLAI	201	9	1122
LIVLAI	201	11	1123
LLKYRAREPV	121	10	1124
LLKYRAREPV	120	11	1125
LLMQDLVQENY	245	11	1126
			1127

Table XIV A
Mape 2 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LMQDLDLVQENY	246	10	1128
LQLVFGIEV	158	9	1129
LQLVFGIEVV	158	10	1130
LVEVTLGEV	45	9	1131
LVFGIEV	160	8	1132
LVFGIEVVEV	160	10	1133
LVFGIEVVEVV	160	11	1134
LVHFILLKY	116	9	1135
LYQENYLEY	250	9	1136
LVTGIGLSY	178	9	1137
MFKTGLLI	196	8	1138
MFKTGLLII	196	9	1139
MPKTGLLIV	196	10	1140
MQDVLVQENY	247	9	1141
NQEIEGPRM	89	9	1142
NQEIEGPRMF	89	10	1143
NQVMPKTGLLI	193	11	1144
PISIILYLIV	171	9	1145
PHISPQGASSF	61	11	1146
PQGASSFSFTI	65	11	1147
PVIFSKASEY	148	10	1148
PVTKAEMLESV	129	11	1149
QLVFGIEV	159	8	1150
QLVFGIEVV	159	9	1151
QLVFGIEVVEV	159	11	1152
OOTASSSSSTLV	36	11	1153
QVMKRTGLLI	194	10	1154
QVMPKTGLLI	194	11	1155
QVPGSDPACY	260	10	1156
RMFPDLSEEF	96	10	1157
ROYPGSDPACY	259	11	1158
SPQGASSF	64	8	1159
SVFIAHPRKLLM	237	11	1160
SVLRNCQDF	138	9	1161
SVLRNCQDF	138	10	1162
TLKIGGEPHI	290	10	1163
TLVEVTLGEV	44	10	1164
VIFSKASEY	149	9	1165
VLIHTIKI	286	8	1166
VLRNQCDFF	139	8	1167
VLRNQCDFF	139	9	1168
VLRNQCDFFPV	139	11	1169
VMPKTGLLI	195	9	1170
VMPKTGLLII	195	10	1171
VPGSDPACY	195	11	1172
VPGSDPACYEF	261	9	1173
VPISHLYI	170	8	1174
VPSHLYI	170	10	1175
VQENYLEY	251	8	1176

Table XIV A
Mage 2_B62_Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
VQENYLEYRQV	251	11	1178
VVEVVPISHLY	166	11	1179
VVPISHLY	169	8	1180
VVPISHLYI	169	9	1181
VVPISHLYILV	169	11	1182
YILVTCGLSY	176	11	1183
YLQLVFGI	157	8	1184
YLQLVFGIEV	157	10	1185
YLQLVFGIEVV	157	11	1186
YVKVLJHTLKI	283	11	1187

Table XIV B
Mage 3_B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
ALSRKVAELV	108	10	1188
ALVETSYV	277	8	1189
ALVETSYVKV	277	10	1190
DIACYEFLW	265	9	1191
DPIGHLYI	170	8	1192
DPIGHLYIF	170	9	1193
DPKKLUTQHIF	241	10	1194
DPKKLUTQHIFV	241	11	1195
ELMEVDPI	165	8	1196
ELSVLEVF	224	8	1197
ELVHFLLKY	115	10	1198
FMLGSVVGKW	134	10	1199
EPTYKAEM	128	8	1200
EVDPIGHLY	168	9	1201
EVDPIGHLYI	168	10	1202
EVDPIGHLYIF	168	11	1203
EVFEGREDSI	229	10	1204
FLWGPRALV	271	9	1205
FPDLESFF	98	8	1206
FQAALSRKV	105	9	1207
FVQENYKLV	250	9	1208
GIELMEVDPI	163	10	1209
GLLGDNQI	188	8	1210
GLLGDNQIM	188	9	1211
GLLIVLAI	200	9	1212
GLLIVLAI	200	10	1213
GPRALVETSY	274	10	1214
GPRALVETSYY	274	11	1215
HISYPPLHEW	298	10	1216
HISYPPLHEWY	298	11	1217
IMVKISGGPHI	289	8	1218
IMPKAGLLI	195	9	1219
IMPKAGLLII	195	10	1220
IMPKAGLJIV	195	11	1221
KISGGPHII	292	8	1222
KISGGPHISY	292	10	1223
KIWEELSIV	220	8	1224
KIWEELSIVF	220	11	1225
KLLTQHIFV	244	8	1226
KVAELVIF	112	8	1227
KVLHIMVKI	285	9	1228
LIVLAI	202	8	1229
LLGDNQIM	189	8	1230
LLIVLAI	201	8	1231
LLIVLAI	201	9	1232
LLKYRAREPV	121	10	1233
LLKYRAREPV	120	12	1234
LLTQHIVQENY	245	11	1235
LMEVDPIGHLY	166	11	1236
LPTTMNYPLW	71	10	1237

Table XIV B
Mage 3 B62 Supermotif Peptides

Sequence	Position	No. of Amino Acids	SEQ ID NO.
LQLVFGIELM	158	10	1238
LVETSYVKV	278	9	1239
LVEVTLGEV	45	9	1240
LVFGIELM	160	8	1241
LVFGIELMEV	160	10	1242
LVIFLLLKY	116	9	1243
MLGSVVGNNW	135	9	1244
MLGSVVGNNWQY	135	11	1245
MPIKAGLLI	196	8	1246
MPIKAGLLII	196	9	1247
MPIKAGLLIV	196	10	1248
MVKISGGPIL	290	10	1249
NQEEEGPSTIF	89	10	1250
NQIMPKAGLLI	193	11	1251
PIGHLYIF	171	8	1252
PQGASSLPLTM	65	11	1253
PVTKAEMMLGSV	129	11	1254
QIMPKAGLLI	194	10	1255
QIMPKAGLLII	194	11	1256
QLVFGIELM	159	9	1257
QLVFGIELMEV	159	11	1258
QVPGSDPACY	260	10	1259
RQVPGSDPACY	259	11	1260
SLPTTMNY	70	8	1261
SLPTTMNYPLW	70	11	1262
SLOLVFGI	157	8	1263
SLOLVFGIELM	157	11	1264
SVVGNWQY	138	8	1265
SVVGNWQYF	138	9	1266
SVVGNWQYFF	138	10	1267
TLVEVTLGEV	44	10	1268
TNNYPLWSQSYY	74	11	1269
TQIFVQENY	247	9	1270
VLIHIMVKI	286	8	1271
VPGSDPACY	261	9	1272
VPGSDPACYEF	261	11	1273
VQENYLEY	251	8	1274
VQENYLEYRQV	251	11	1275
VVGNNWQYF	139	8	1276
VVGNNWQYFF	139	9	1277
VVGNNWQYFFPV	139	11	1278
WQYFFPVVI	143	8	1279
WQYFFPVIF	143	9	1280
YIFATCLGLSY	176	11	1281
YPLWSQSYY	77	8	1282
YPLWEWV	301	8	1283
YVKVLHIM	283	8	1284
YVKVLHIMV	283	9	1285
YVKVLHIMVKI	283	11	1286

Table XV A
Mage 2A01 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0101	SEQ ID NO.
ASSFSTTINY	68	10	0.1700	1287
GASSEFTTINY	67	11	0.0047	1288
GGEPHISY	294	8	-0.0021	1289
IFSKASEY	150	8	0.0023	1290
LMDLIVQENY	246	10	0.0450	1291
MDLVQENY	247	9	1.5000	1292
PGSDPACY	262	8	-0.0021	1293
PRALIETSY	275	9	-0.0006	1294
SFSTTINY	70	8	-0.0021	1295
SFSFTTINY	69	9	0.0430	1296
VQENYLEY	251	8	-0.0021	1297
VTCLGLSY	179	8	0.2000	1298
VVEVVPISHY	166	11		1299

Table XV B
Mage 3 A01 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*P0101	SEQ ID NO.
ASSLPTTMNY	68	10	2.6000	1300
ATCLGLSY	179	8	0.1100	1301
EVDPIGHLY	168	9	18.0000	1302
GASSLPTTMNY	67	11	0.0390	1303
GSVYVGNNWQY	137	9	0.0500	1304
IFATCLGLSY	177	10	0.0020	1305
ISGGPHISY	293	9	0.0370	1306
KISGGPHISY	292	10	0.0011	1307
LGSVVGNNWQY	136	10	0.0020	1308
LMEVDPIGHLY	166	11	7.5000	1309
LTQHIVQENY	246	10	0.2600	1310
PGSDPACY	262	8	-0.0021	1311
PRALVETSY	275	9	0.0011	1312
SSLPTTMNY	69	9	0.0550	1313
TMNYPLWSQSY	74	11	0.0830	1314
VQENYLEY	251	8	-0.0021	1315

Table VI
Mage 2 A03 Motif Peptides with Binding Data

SEQ ID NO.	A*	Position	No. of Amino Acids	Value
AAADSPSPH		55	55	0.0003
ACYEFLWGPR		267	10	0.0032
ACYEFLWGPR^A		267	11	1316
AIDSPSPH		56	8	1317
AIEGDCAPEEK		210	11	1318
AIAGEGDCA		207	10	1319
AISRKMVELVII		108	11	1320
ALGLGLVGAAQAA		22	9	1321
ALGLGLVGAAQAA		22	11	1322
ALIETSYVK		277	9	1323
ASEYQLQLVF		154	9	1324
ASSFSTINY		68	10	1325
ATEEQQTIA		32	8	1326
DFFPVIFSK		145	9	1327
DFFPVIFSKA		145	10	1328
DLESEFOQA		100	8	1329
DLESEFOQA		100	9	1330
DLVQENYLEYR		249	10	1331
DLVQENYLEYR		249	11	1332
DSVFAIIPR		236	8	1333
DSVFAIIPK		236	9	1334
EALGLYGA		21	8	1335
EALGLVGAAQAA		21	10	1336
EDSVFAIIPR		235	9	1337
EDSVFAIIPRK		235	10	1338
EFFLWGRA		270	8	1339
EFOQAAISR		104	8	1340
EFOQAAISR		104	9	1341
EFOQAAISR^K		104	9	1342
EIDGDCAPEEK		212	9	1343
EGGLEARGEAA		14	9	1344
EGREDSDVF		232	8	1345
EGREDSDVF		232	9	1346
EGREDSDVFII		232	10	1347
ELSMILIVFEGR		224	8	1348
ELVHFLLKK		115	9	1349
ELVHFLLKKY		115	10	1350
EMLESVLR		134	8	1351
ESFOQAAISR		102	10	1352
ESFOQAAISR^K		102	11	1353
ESVLRNCODF		137	10	1354
ESTSYVKVLI		280	9	1355
ESTSYVKVLI		280	10	1356
EVFEGREDSVF		229	11	1361
EVTLGEVPA		47	9	1362
EVTLGEVPA		47	10	1363
EVVEVVPISH		165	10	1364
EVVPISHLY		168	9	1365

Table XVI A
Motif 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
FFPVIFSK	146	8	0.0003	1366
FFPVIFSKA	146	9	0.0003	1367
FLLKYRAR	119	8	0.0003	1368
FLLKYRAR	119	9	0.0003	1369
FSTTINYTLWR	71	11	0.0110	1370
GASSFSTTINY	67	11	0.0110	1371
GDCAPEEK	213	8	0.0780	1372
GDNQVMPK	191	8	0.0003	1373
GGEPHISY	294	8	0.0003	1374
GIEARGEA	15	8	0.0002	1375
GILGDNQVMPK	188	11	0.0002	1376
GLIIVLVA	200	8	0.0003	1377
GLIIVLVAIA	200	11	0.0003	1378
GLVGAQAPPA	24	9	0.0003	1379
GSDPACYEF	263	9	0.0002	1380
GSSNQEEFGR	86	11	0.0002	1381
HCKPEEGLEA	9	10	0.0003	1382
HCKPEEGLEAR	9	11	0.0003	1383
HFLLLKYRA	118	8	0.0016	1384
HFLLLKYRAR	118	9	0.0016	1385
HISYPPLII	298	10	0.0014	1386
HISYPPLIHER	298	8	0.0014	1387
HISYPPLIERA	298	10	0.0074	1388
HSPQGASSSF	63	9	0.0002	1389
HTLKIGGIPHI	289	10	0.0002	1390
IALEGDCA	209	8	0.0002	1391
IFSKASEY	150	8	0.0002	1392
IGGEPHISY	293	9	0.0002	1393
HAIEGDCA	208	9	0.0002	1394
HVLALIA	203	8	0.0002	1395
ILVTCLGLSY	177	10	0.0036	1396
ISRKAMVELVIT	109	10	0.0002	1397
ISRKAMVELVIF	109	11	0.0002	1398
ISYPPLIHER	299	9	0.0340	1399
ISYPPLIERA	299	10	0.0002	1400
KAEMLESVLR	132	10	0.0002	1401
KASEYLOLVF	153	10	0.0002	1402
KIGGEPHISY	292	10	0.0002	1403
KMVELVIF	112	8	0.0002	1404
KTGLLIIVLA	198	10	0.0002	1405
KVLIHHTLK	285	8	0.0053	1406
LAIIALEGDCA	206	11	0.0053	1407
LGDNOVMPK	190	9	0.0002	1408
LGLVGAAQAPPA	23	8	0.0002	1409
LGLVGAAQAPPA	23	10	0.0003	1410
LIETSYVK	278	8	0.0003	1411
LIETSYVKVLII	278	11	0.0004	1412
LIIVLVAIA	202	9	0.0004	1413
LLGDNQVMPK	189	10	0.0093	1414

Table XVI A
Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
LIIIVLAIKA	201	10		1416
LLKRYRAR	120	8	-0.0009	1417
LLMODLVQENY	245	11		1418
LMQDLVQENY	246	10		1419
LSMLEVFGGR	225	10		1420
LVEVTLGIVTA	45	11		1421
LVGAQAPAA	25	8	0.0290	1422
LVIIFULLK	116	8	0.0430	1423
LVIIFULLKY	116	9	0.0260	1424
LVIIFULLKRY	116	10		1425
LVIIFULLKRYA	116	11		1426
LVQENYLFF	250	9		1427
LVQENYLYFRR	250	10	0.0027	1428
LVTCLGLSY	178	9		1429
MFTDLESEF	97	9	0.0002	1430
MFPDLESEFQAA	97	11		1431
MLEVFEGR	227	8	-0.0009	1432
MVELVIFULLK	113	11	0.0200	1433
NCQDFPFWIF	142	10	0.0002	1434
PAADSPSPH	54	10		1435
PACYEFLVGPR	266	11	-0.0009	1436
PATEEQQTAA	31	9		1437
PDELESEFQAA	99	9	0.0003	1438
PDELESEFQAA	99	10	0.0003	1439
PGSDPACY	262	8		1440
PGSDPACYEF	262	10		1441
PLEQRSSQI	2	8		1442
PLEQRSSQICK	2	10		1443
PLIUERALR	303	8	-0.0009	1444
PPPPISPGQA	59	10		1445
PVIFSKASEY	148	10	0.0160	1446
QAPATEEQQTA	29	11		1447
QDFFPVWIF	144	8		1448
QDFFPVIFSK	144	10		1449
QDFFPVIFSKA	144	11		1450
QDLVQENY	248	8		1451
RALIETSY	276	8		1455
RALIETSYVK	276	10	0.0200	1456
RAREPVTK	125	8	-0.0009	1457
RAREPVTKA	125	9		1458
RGEALGLGVGA	19	10		1459
RMFPDLESEF	96	10	0.0003	1460
SDPACYEF	264	8	0.0002	1461
SFSSTINY	70	8		1462
SMLEVFGGR	226	9		1463
SSFSSTINY	69	9	0.0020	1464
SSNQEEEGPR	87	10	0.0002	1465

Table XVI A
Mage 2 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
SITINYTLWR	72	10	0.0014	1466
SVFALIPRK	237	8	0.1410	1467
SVLRNCODF	138	9	0.0002	1468
SVLRNCOOFF	138	10	0.0002	1469
TGLLIVLVA	199	9		1470
TINYTLWR	74	8	0.0140	1471
TLGEVPA	49	8		1472
TLKIGGEPH	290	9		1473
TSYVKVLH	281	8		1474
TSYVKVLH	281	9	0.5900	1475
TTINYTLWR	73	9	0.0890	1476
VEIGREIDSVF	230	10		1477
VEIGREIDSVFA	230	11		1478
VIFSKASEY	149	9	0.0810	1479
VLRNQCDFF	139	8	0.0002	1480
VLRNQCDFF	139	9		1481
VTCLGLSY	179	8		1482
VTLGIEVPA	48	8		1483
VTLGIEVPA	48	9		1484
VVEVVPISII	166	9		1485
VVEVVPISILY	166	11		1486
VVPISHLY	169	8		1487
WGPRALIETSY	273	11		1488
YILVTCGLSY	176	11		1489
YVKVLHITLK	283	10	0.0033	1490

Table XVI B
Map3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
ΔA1SLRKVΔ	107	8	0.0032	1491
ACYEFLWGPR	267	10	0.0006	1492
ACYEFLWGPRΔ	267	11	0.0006	1493
AGLIIIVLA	199	9	0.0006	1494
ΔIAREGDCΔ	207	10	0.0003	1495
ΔLGLVGAQA	22	9	0.0003	1496
ΔLGLVGAQAPΔ	22	9	0.0003	1497
ΔLSRKVAELVII	108	11	0.0270	1498
ΔLVETSYVK	277	9	0.0009	1499
ASSLPTIMNV	68	10	0.0009	1500
ASSSLQLVF	154	9	0.0011	1501
ΔTCLGLSΔY	179	8	-0.0004	1502
ΔVEEQEΔAA	32	8	-0.0003	1503
DLESEFQΔA	100	8	0.0003	1504
DLESEFQΔA	100	9	0.0003	1505
DSILGDIK	236	8	0.0003	1506
DSILGDIK	236	9	0.0003	1507
EALGLVGAΔA	21	8	0.0003	1508
EALGLVGAQQA	21	10	0.0003	1509
EDSILGDIK	235	9	0.0003	1510
EDSILGDPKK	235	10	0.0003	1511
EFLWGPRΔ	270	8	0.0002	1512
EFOQAALSR	104	8	0.0002	1513
EFOQAALSRK	104	9	0.0002	1514
EFOQAALSRKVΔ	104	11	0.0002	1515
EGDCAPEHK	212	9	0.0002	1516
EGLEARGEΔA	14	9	0.0003	1517
ELMEVDPGH	165	10	0.0003	1518
ELSVLLEVΔF	224	8	0.0003	1519
ELSVLLEVFEGR	224	11	-0.0009	1520
ELVIFILLK	115	9	0.0045	1521
ELVIFILLKY	115	10	0.0066	1522
ELVIFILLKYR	115	11	0.0011	1523
ESIEFOQAALSR	102	10	0.0002	1524
ESIEFOQAALSRK	102	11	0.0002	1525
ETSYYKVLI	280	9	0.0002	1526
ETSYYKVLIΔI	280	10	0.0002	1527
EVDPIGHLY	168	9	0.0002	1528
EVDPIGHLYIF	168	11	0.0003	1529
EVTLGEPVΔA	47	9	0.0003	1530
EVTLGEPVΔA	47	10	0.0003	1531
FATCIGLΔSY	178	9	0.0003	1532
FFPVIFSK	146	8	0.0003	1533
FFPVIFSKA	146	9	0.0003	1534
FLLKYRAΔR	119	8	0.0003	1535
FVQENYLΔF	250	9	0.0009	1536
FVQENYLΔF	250	10	0.0009	1537
GASSLTTTMNY	67	11	0.0009	1538
GDCΔPEEK	213	8	0.0009	1539

Table XVI B
Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
GDNQIMPK	191	8	0.0003	1541
GDNQIMPKA	191	9	0.0003	1542
GDPKKLTLTQII	240	10		1543
GDPKKLTLTQIF	240	11		1544
GGPHISYPPLII	295	11		1545
GLEARGEA	15	8	0.0003	1546
GLLGDNQIMPK	188	11	0.1300	1547
GLLIIVLA	200	8		1548
GLLIIVLAIA	200	11		1549
GLVGQAQAPPA	24	9	0.0003	1550
GSDPACYEF	263	9		1551
GSVVGNNWQY	137	9		1552
GSVVGNNWQYWF	137	10		1553
GSVVGNNWQYFF	137	11		1554
HCKPPEEGLEA	9	10	0.0003	1555
HCKPPEEGLEAR	9	11	0.0003	1556
HFLLLKYR	118	8	0.0016	1557
HFLLLKYRA	118	9	0.0014	1558
HFLLLKYRAR	118	10		1559
HFVQENYLEY	249	10		1560
HFVQENYLEYR	249	11		1561
HISYPPLII	298	8		1562
IMVKISGGPHI	289	10		1563
IAREGIDCA	209	8		1564
IFATCLGLSY	177	10		1565
IGILYIFA	172	8		1566
IAREGIDCA	208	9		1567
IIVLAIIA	203	8		1568
IIVLAIAR	203	9		1569
ISGGPHISY	293	9	0.0003	1570
IVLAIAR	204	8	0.0053	1571
KAGLLIVLA	198	10		1572
KASSHQLVF	153	10		1573
KISGGPHISY	292	10		1574
KVAELVIF	112	8		1575
KVLHIMVK	285	8	0.0580	1576
LAHAREGIDCA	206	11		1577
LGDNQIMPK	190	9		1578
LGDNQIMPKA	190	10		1579
LGDPKKLLTQII	239	11	0.0003	1580
LGLVGAQAA	23	8		1581
LGLVGAQAPA	23	10	0.0003	1582
LGSVVGNNWQY	136	10	0.0003	1583
LGSVVGNNWQYF	136	11		1584
LIIVLAIIA	202	9		1585
LIIVLAIAR	202	10	0.0280	1586
LLGDNQIMPK	189	10	0.0200	1587
LLGDNQIMPKA	189	11		1588
LLIIVLAIIA	201	10		1589
LLIIVLAIAR	201	11	0.0021	1590

Table XVI B
Mage 3 A03 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
LLLKYRAR	120	8	-0.0009	1591
LLTQHVFQENY	245	11	0.0002	1592
LMEVDPIGH	166	9	0.0002	1593
LMEVDPIGHLY	166	11	0.0002	1594
LSRKVAELVII	109	10	0.0002	1595
LSRKVAELVIF	109	11	-0.0006	1596
LSVLEVTEGR	225	10	-0.0006	1597
LTQHVFQENY	246	10	0.0003	1598
LVEITSVK	278	8	-0.0004	1599
LVEITSVKVLI	278	11	1600	1600
LVEVTLGIVPA	45	11	1601	1601
LVGAQAPAA	25	8	0.0290	1602
LVIFFLLK	116	8	0.0430	1603
LVIFLLKY	116	9	0.0260	1604
LVIFLLKYR	116	10	1605	1605
LVIFLLKYRA	116	11	1606	1606
MLGSVVGNNWQY	135	11	1607	1607
MVKISGGPHI	290	9	0.0003	1608
PACYEFWLWGR	266	11	-0.0009	1609
PATEFOEA	31	8	0.0003	1610
PATEFOEA	31	9	0.0003	1611
PDLESEFQAA	99	9	0.0003	1612
PDLESEFQAA	99	10	0.0003	1613
PDPHQSPQGA	59	10	0.0003	1614
PGSDIACY	262	8	0.0003	1615
PGSDPACYEF	262	10	1616	1616
PIGHLYIF	171	8	1617	1617
PIGHLYIF	171	9	1618	1618
PLEQRSQLI	2	8	1619	1619
PLIQRSQLICK	2	10	1620	1620
PLIIEWVLK	303	8	1621	1621
PSTIPDIESFF	95	11	1622	1622
QVALRSRKVA	106	9	1623	1623
QAPATHEQEAA	29	10	1624	1624
QAPATIEFQEAA	29	11	1625	1625
QVPGSDPAA	260	8	1626	1626
QVPGSDIACY	260	10	1627	1627
RALVETSY	276	8	1628	1628
RALVETSYVK	276	10	1629	1629
RAREFVTK	125	8	1630	1630
RAREFVTKA	125	9	1631	1631
RGEALGLVGA	19	10	1632	1632
SDPACYEF	264	8	1633	1633
SGGPHEISY	294	8	1634	1634
SILGDPKK	237	8	1635	1635
SLPTTMNY	70	8	1636	1636
SSLPTTMNY	69	9	1637	1637
SSSQLQLVF	155	8	1638	1638
STFDLSEF	96	10	1639	1639
SVLEVFEGR	226	9	1640	1640

Mate 3 A03 Motif Peptides with Binding Data

Table XVIIB

Sequence	Position	No. of Amino Acids	A*0301	SEQ ID NO.
SVVGNWQYF	138	8	0.0002	1641
SVVGNWQYFF	138	9	0.0085	1642
TFPDLESEF	97	10	0.0002	1643
TFPDLESEFQAA	97	9	0.0002	1644
TLGEVPA	49	11	0.0002	1645
TMNYPLWSQSY	74	8	0.0002	1646
TSYVKVLII	281	11	0.0002	1647
TSYVKVLIIH	281	8	0.0002	1648
VAEIVIIFULK	113	9	0.0002	1649
VDPIGILY	169	11	0.0002	1650
VDPIGILYIF	169	10	0.0002	1651
VDPIGILYIF^	169	11	0.0002	1652
VGNWQYFF	140	8	0.0002	1653
VLEVFEGR	227	8	0.0002	1654
VTLGEVPA	48	8	0.0002	1655
VTLGEVPA	48	9	0.0002	1656
VVGNWQYFF	139	8	0.0002	1657
VVGNWQYFF	139	9	0.0002	1658
WGPRALVETSY	273	11	0.0002	1659
YFPVIFSK	145	9	0.0002	1660
YFPVIFSK	145	10	0.0002	1661
YIFATCLGILSY	176	11	0.0002	1662
YVKVLUHIMVK	283	10	0.0002	1663
			0.0020	1664

Table XVIIA
Mage 2 All Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
AADSPPH	55	9	0.009	1665
ACYEFLWGPR	267	10	0.0035	1666
ADSPSPH	56	8		1667
AIEGDCAPEEK	210	11	0.0007	1668
AISRKMAVLLVII	108	11		1669
AIETSYVK	277	9	0.1900	1670
ASSESTINY	68	10	0.0260	1671
DFFPVHSK	145	9	0.0022	1672
DLVQENYLEY	249	10		1673
DLVQENYLEYR	249	11	0.0018	1674
DSVFAIPR	236	8	0.0005	1675
DSVFAIPRK	236	9	0.0025	1676
EDSVAIPR	235	9		1677
EDSVAIPRK	235	10		1678
EFOAAISR	104	8		1679
EFOAAISRK	104	9	0.0002	1680
EGIDCAPEK	212	9	0.0001	1681
EGREDSVFAH	232	10		1682
ELSMILEVFEGR	224	11	0.0008	1683
ELVHFLLK	115	9	0.0011	1684
ELVHFLLKY	115	10	0.0003	1685
ELVIFLKKYR	115	11	0.0031	1686
EMLESVLR	134	8	-0.0003	1687
ESFFQAAISR	102	10	0.0002	1688
ESFFQAAISRK	102	11	0.0004	1689
ETSYYKVLLI	280	9		1690
ETSYYKVLLI	280	10		1691
EVVEVYPSH	165	10	0.0002	1692
EVVPISLY	168	9	0.0002	1693
FPPVIESK	146	8		1694
FLLIKYRAR	119	9		1695
FSTTINYTLWR	71	11	0.0170	1696
GASSSTINY	67	11		1697
GDCAPEEK	213	8		1698
GDNQVMPK	191	8		1699
GGEPHSY	294	8		1700
GLGIDNQYMPK	188	11	0.0047	1701
GSSNQEEGP	86	11	-0.0002	1702
HICKPEGLEAR	9	11		1703
IPLLKYR	118	8		1704
IPLLKYRAR	118	10	0.0002	1705
IISYPPLH	298	8		1706
IISYPPLHR	298	10	0.0018	1707
ITLKIGGEPH	289	10		1708
IFSKASEY	150	8		1709
IGCEPHSY	293	9		1710
ILVTCGLSY	177	10	0.0002	1711
ISRKAVELVII	109	10	0.0002	1712
ISYPPLIER	299	9	0.0280	1713
KAEMLESVL	132	10	0.0009	1714

Table XVIIA
Mage 2 All Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
KIGGEPHSY	292	10		1715
KVLIHTLKK	285	8	0.0100	1716
LGDNQVMPK	190	9	0.0061	1717
LIETSYVK	278	8	0.0027	1718
LIETSYVKVLH	278	11		1719
LLGDNQVMPK	189	10	0.0014	1720
LLKTYPRAR	120	8	-0.0004	1721
LMQDLVQENY	245	11		1722
LMQDLVQENY	246	10		1723
LSMLEVEFGR	225	10	0.0001	1724
LVIFFLIK	116	8	0.1500	1725
LVIFFLIKY	116	9	0.0100	1726
LVIFFLIKYR	116	10	0.0022	1727
LVQENYLYF	250	9		1728
LVQENYLYF	250	10	0.0089	1729
LVTCLGLSY	178	9		1730
MLEVFEGR	227	8	-0.0004	1731
MVELVHFLLK	113	11	0.0120	1732
PAADSPSPPII	54	10		1733
PACYEFLWGPY	266	11	-0.0002	1734
PGSDPACY	262	8		1735
PLEQRSQLI	2	8		1736
PLEQRSQLICK	2	10	0.0002	1737
PLIEFLALR	303	8	-0.0004	1738
PVFSKASEY	148	10	0.0033	1739
QDFFPVIFSK	144	10	0.0083	1740
QDLVQENYLY	248	8		1741
QDLVQENYLY	248	11		1742
QVFGSDPACY	260	10		1743
RALIETSY	276	8		1744
RALIETSYK	276	10	0.0750	1745
RAREFVTK	125	8	-0.0003	1746
SFSTTINY	70	8		1747
SMLEVPEGR	226	9	0.0220	1748
SNOQEFGPR	88	9	0.0001	1749
SSFSITINY	69	9		1750
SSNQEEFGPR	87	10	0.0002	1751
STTINYTLWR	72	10	0.0910	1752
SVFAHPRK	237	8	0.0810	1753
TINYTLWR	74	8	0.0559	1754
TLKIGGEPIL	290	9		1755
TSYVKVLH	281	8	0.0066	1756
TSYVKVLH	281	9	1.1000	1757
TTINYTLWR	73	9	0.0330	1758
VIFSKASEY	149	9		1759
VTCLGLSY	179	8	0.0100	1760
VVEVVPISHI	166	9		1761
VVEVVPISHI	166	11		1762
VVPISHLY	169	8		1763
WGPRALIETSY	273	11		1764

Table XVIIA
Mage 2 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
YILVTCLGSLV YVKVLNIRLK	176 283	11 10	0.0160	1765
				1766

Table XVII
Mage 3 A11 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
ACYEFLWGPR	267	10	0.0035	1767
ALSRKVAVLVI	108	11	0.1700	1768
ALVETSYVK	277	9	0.0330	1769
ASSLPTTMNY	68	10	-0.0003	1770
ATCLGLSY	179	8	-0.0002	1771
DSILGDPK	236	8	0.0002	1772
DSILGDPKK	236	9	0.0002	1773
EDSILGDPK	235	9	0.0002	1774
EDSILGDPKK	235	10	0.0002	1775
EFOQAALSR	104	8	0.0001	1776
EFOQAALSRK	104	9	0.0001	1777
EGDCAPEEK	212	9	0.0001	1778
ELMEVIDPGH	165	10	0.0002	1779
ELSVIEVFEGR	224	11	0.0023	1780
ELVIFILLK	115	9	0.0011	1781
ELVIFILLKY	115	10	0.0003	1782
ELVIFILLKYR	115	11	0.0031	1783
ESEFQAAALSR	102	10	0.0002	1784
ESEFQAAALSRK	102	11	0.0004	1785
ETSYVKVLIH	280	9	0.0009	1786
ETSYVKVLIHI	280	10	0.0009	1787
EVDPIGHLY	168	9	0.0004	1788
FATCLGLSV	178	9	0.0004	1789
FFPVIEFK	146	8	0.0002	1790
FLLIKYRAR	119	9	0.0002	1791
FVQINYLEY	250	9	0.0012	1792
FVQENYLFLYR	250	10	0.0012	1793
GASSLYTTMNY	67	11	0.0570	1794
GDCAPEEK	213	8	0.0002	1795
GDNQIMPK	191	8	0.0002	1796
GDPKKLTLQI	240	10	0.0002	1797
GGPHISYPPLI	295	11	0.0002	1798
GLLGDNQIMPK	188	11	0.0002	1799
GSVVGNNWQY	137	9	0.0002	1800
HCKPREFGEAR	9	11	0.0002	1801
IFLLLKYR	118	8	0.0002	1802
IFLLLKYRAR	118	10	0.0002	1803
HFVQENYLEY	249	10	0.0004	1804
HFVQENYLEYR	249	11	0.0011	1805
HISYPPLI	298	8	0.0002	1806
IMVKISGGPH	289	10	0.0002	1807
IFATCLGLSY	177	10	0.0002	1808
IVLAIIAR	203	9	0.0002	1809
ISGGPHISY	293	9	0.0002	1810
IVLAIIAR	204	8	0.0037	1811
KISGGPHISY	292	10	0.0019	1812
KVLIIIMVK	285	8	0.0012	1813
LGDNQIMPK	190	9	0.0014	1814
LGDPKKLTLQI	239	11	0.0015	1815
LGSVVGNNWQY	136	10	0.0012	1816

Map 3 A11 Motif Peptides with Binding Data

Table XVII B

Sequence	Position	No. of Amino Acids	A*1101	SEQ ID NO.
LIVLALLAR	202	10	0.0021	1817
LIGDDNQIMPK	189	10	0.0110	1818
LLIVLALLAR	201	11	0.0056	1819
LLKYRARR	120	8	-0.0004	1820
LTQHQFVQENY	245	11		1821
LMEVDPIGH	166	9	0.0001	1822
LMEVDPIGHLY	166	11		1823
LSRKVAELVH	109	10	0.0002	1824
LSVLEVFGGR	225	10	0.0030	1825
LTOHFVQENY	246	10	0.0002	1826
LVEISYVK	218	8	0.0014	1827
LVEISYVKLVII	278	11		1828
LVIFFLLK	116	8	0.1500	1829
LVIFFLKKY	116	9	0.0100	1830
LVIFFLKKYR	116	10	0.0022	1831
MLGSVGVNWQY	135	11		1832
MNYPLWSQSY	75	10	0.0002	1833
MVKISGGPHI	290	9	0.0002	1834
PACYEFLWGPRL	266	11	-0.0002	1835
PGSDPACY	262	8		1836
PLEORSOII	2	8		1837
PLEQRSQLCK	2	10	0.0002	1838
PLIHEWVLR	303	8	-0.0003	1839
QVIGSDPACY	260	10		1840
RALVETSY	276	8		1841
RALVETSYK	276	10	0.1100	1842
RAREPTVK	125	8	-0.0003	1843
SGGPHISY	294	8		1844
SILGDPKK	237	8		1845
SLLTTMNY	70	8		1846
SSLTTMNY	69	9		1847
SVLEVFGGR	226	9	0.1400	1848
SVVGRNWQY	138	8		1849
TMNYPPLWQSQY	74	11		1850
TSYVKVLI	281	8		1851
VAELVHFLLK	113	9		1852
TSYVKVLI	281	11	0.0011	1853
VDPIGHLY	169	8		1854
VLEVFGGR	227	8		1855
WGPRALVETSY	273	11		1856
YFFPVIFSK	145	9		1857
YIFATCLGLSY	176	11	0.0270	1858
YVKVLHIMVK	283	10	0.0061	1859

Table XVIII A**Mage 2 Δ24 Motif Peptides with Binding Data**

Sequence	Position	No. of Amino Acids	Δ*2401	SEQ ID NO.
CYEFLWGPRAL	268	11	0.0004	1860
EELWGPRAL	270	9	0.0006	1861
EFLWGPRALI	270	10	0.0097	1862
FYLQLVFGI	156	9	3.5000	1863
IFSKASEYL	150	9	0.0230	1864
IFSKASEYLQL	150	11	0.0950	1865
IWEELSMI	221	8	0.0007	1866
IWEELSMIWF	221	11	0.0170	1867
KMVELVIF	112	8	0.0005	1868
KMVELVIFL	112	9	1869	
KMVELVIFLL	112	10	1870	
KMVELVIFLLI	112	11	1871	
LMQDLYQENYL	246	11	0.1200	1872
LWGPRALI	272	8	0.0086	1873
LYILVTCI	175	8	0.0140	1874
LYILVTCIGL	175	10	0.0140	1875
MFPDLFSEF	97	9	0.0140	1876
RMFPDLFSEF	96	10	0.0016	1877
SFSTTINYTL	70	10	0.0150	1878
SFSTTINYTLW	70	11	0.0280	1879
SYPLLIERAL	300	10	0.0003	1880
SYVKVLRHITL	282	10	0.1600	1881
VFAIPRKLI	238	8	0.0005	1882
VFAIPRKLL	238	9	0.0006	1883
VFEGREDSVF	230	10	0.0004	1884
VMPKTCILL	195	8	-0.0004	1885
VMPKTGILL	195	9	0.2300	1886
VMPKTGILLII	195	10	0.0580	1887

Table XVIII B
Mage 3 Δ24 Motif Peptides with Binding Data

Sequence	Position	No. of Amino Acids	A*2401	SEQ ID NO.
CYFFILYGIRAL	268	11	0.0004	1888
EFLWGRPAL	270	9	0.0006	1889
EMLGSVVGPNW	134	10	0.0017	1890
HIVQENYL	249	8	-0.0004	1891
IMVKISGGMII	289	11	1892	
IFATCLGL	177	8	0.0120	1893
IFSKASSSL	150	9	0.0160	1894
IFSKASSSLQL	150	11	0.0910	1895
IMPKAGLL	195	8	0.4200	1896
IMPKAGLLI	195	9	0.0500	1897
IMPKAGLLII	195	10	1898	
IWFELLSVL	221	8	-0.0004	1899
IWEELSVLEV	221	11	0.0260	1900
LMEVDPIGH	166	10	1901	
LYIFATCCL	175	8	0.0140	1902
LYIFATCGL	175	10	0.0480	1903
NWQYFFFV	142	9	0.5300	1904
NWQYFFFVIF	142	10	0.0170	1905
QYFFFVIF	144	8	0.1200	1906
SYDGILGDNQI	185	11	0.0026	1907
SYPLHIEW	300	8	0.0420	1908
TFDLESEF	300	10	0.5900	1909
VFEGREDSI	97	9	0.0049	1910
VFEGREDSI	230	9	-0.0004	1911
VFEGREDSI	230	10	-0.0005	1912

Table 2 DR Super Motif Peptides with Binding Data

Mage 2 DR Super Motif Peptides with Binding Data

Table XIXA

Core Sequence	Exemplary Sequence	Position	DR1	DR2w8I	DR2w2R2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
VTCLGLSYD LIFERALREG VITOSDPACY VLHIIITLKIG	YILVTCGLGSYDGLL YPTLIFERALREGEE- YRQVIGSDPACYEFL YVKVLIHITLKICGEP	178 303 260 285									1963 1964 1965 1966

Mage2 DR Super Motif Peptides with Binding Data

Table XIXA

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VTCIGLSYD	YILVTCIGLSYDGULL						1963
LINERALREG	YPPLIHERALREGHE-						1964
VPGSDPACY	YRQVPOSDPACYEFL						1965
VLIIIHLKIG	YVKVLIHLTKIGGEP						1966

Mage 3 DR Super Motif Peptides with Binding Data

Table XIX B
Mage 3 DR Super Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	DRw53	SEQ ID NO.
VHFLLLKRYR	AELVHFLLLKRYRARE							1967
LIVLVAIA	AGLILIVLAIAREG	-0.0026						1968
LYGAQATAT	ALGIVGAQAAPATEFQ	-0.0011						1969
LSYIGLLGD	CLGLSYIDGLLGDNQI	-0.0011						1970
LGDNQIMPK	DGLLGDINQIMPKAGI	-0.0011						1971
IWEELSVLE	EELKWEELSVLEVF							1972
WGPRALVET	EFLWGPRALVETSYV							1973
WEELSLEV	EKIWEELSLEVFEVG							1974
LFYRQVNGS	ENVLEYRQVPGSDPA							1975
FQAAALSRKV	ESEFOAALSRSKVAEL							1976
LGEWPAEES	EVTLGVEWPAEESPDP							1977
VIFSKASSS	FFPVIFSKASSSSLQLL							1978
IFSKAKSSL	FFPVIFSKASSSSLQLV							1979
LGLVGAQAP	GFAALGVGAQAPATE							1980
YIFATCGLL	GILYIFATCGLGSYD							1981
LMEVDPIGH	GIELMEVDPIGHLYI							1982
IVLAIAAR	GILLIIVLAIAREGDI							1983
ISYPHLIEW	GPHISYPHLIEWVLR							1984
LLKYRAREP	HFLLLKYRAREPVTK							1985
IFATCGLS	HLYIFATCGLGSYDG							1986
MEVDPIGH	IELMEVDPIGHLYIF							1987
LYIFATCGL	IGILYIFATCGLGSY							1988
MLGSVGNW	KAEMLGSVGVGNWQYF							1989
LJIVLVAII	KAGLLIVLAIARE							1990
LTIQIFVQEN	KKLLTQHQVQENYLE							1991
VPAEESPDP	LGEVTPAESTDPPOS							1992
VGAQAPATE	LGLVGAQAPATEEQE							1993
VLAIAREG	LIVLVAIAREGDCA							1994
IVLAIARE	LJIVLVAIAREGDCA							1995
YRAREPVTK	LLKYRAREPVTKAEM							1996
VEGIEELMEV	LQLVFGIELMEVDPI							1997
VTLGEVPAAA	LVEVTGEGVIAESP							1998
MPKAGLLII	NQIMPKAGLLIIVLA							1999
YFFPVIFSK	NWQYFPVIFSKASS							2000
FPDLESEFQ	PSTFDLESEFQAAAL							2001
FSKASSSLQ	PVIFSKASSSSLQLV							2002
LSRKVAELV	QAAASRKVAELVHFL							2003
VOENYLEYR	QHFVQENYLEYRQVP							2004
FGEHEMVEVD	QLVFGIELMEVDPIG							2005
FPPVIFSKAS	QYFPVIFSKASSSL							2006
VETSYVKVL	RALVETSYVKLHIM							2007
VTKAEMLGS	REPVTKAEMLGSVVG							2008
LVEVTGEV	SSTLVETVLGEVPA							2009
LVHFLLLK	VAELVHFLLLKRYRAR							2010
IGILYIFAT	VDPIGHLYIFATCLG							2011
IELMEVDPI	VFGIELMEVDPIGIL							2012
WQYFPVIF	VGNWQYFPVIFVSKA							2013
LSVLEVEFEG	WEEELSVLEVEFRED							2014
LWGPRALV	YEFLWGPRALVETSY							2015
LHEWVLREG	YPLLIEWVLREGEE-							2016

Mage 3 DR Super Motif Peptides with Binding Data

Motif Pentides with Bimodal Activity

Mage 3 DR Super Motif Peptides with Binding Data**Table XIX B**

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
VPGSDPACY VLHIMVKIS	YRQVPGSDPACYEFL YVKVLIIMVKISGGP						2017 2018

Table XXaA
Mape2 DR3 Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w2B1	DR2w2B2	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
L.SYDGLLGD IWEELSMLE LESEFQAAI MFPDIESEF IEGDCAPIEE IAIEGDCAP LVQENYLEY FGIEVVEVV LMQDLVQEN LLGDNQVMP	CLGLSYDGLLGDNQV EEKIWEELSMLEVF FPDLESEFQAAISRK GPMEPDLESEFQAA HAEIGDCAPEEKW LAIAIEGDCAPEEK MQDLVQENYLEYRQV QLVFGIEVVVEVVPIS RKLLMMDLIVQENYLE YDGLLLGDNQVMPKTC	183 220 100 96 210 208 249 161 245 188									2019 2020 2021 2022 2023 2024 2025 2026 2027 2028

Table 3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LSYDGLLGD IWFELSMLE LESEFQAAI MFIDLESEF IEGDCAPEE IAIEGDCAP LYQENYLFLY FGIEVVEVV LMQDLVQEN LLGDNQVMP	CLGLSYDGLLGDNQV EEKIWEELSMLEVFE FPDLESEPAQAIISKK GPRMFFPDLESEFQAA IIAIEGDCAPEEKW LAIAIEGDCAPEEK MQDLVQENYLEYRQV QLVFGIEVVEVVIFS RKLLMQDLVQENYLE YDGLGDNQVMPKTG	2019 2020 2021 2022 2023 2024 2025 2026 2027 2028					

Table XXXB
Table 3 DR3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	Position	DR1	DR2w201	DR2w202	DR3	DR4w4	DR4w15	DR5w11	DR5w12	SEQ ID NO.
LSYDGILGD IWEELSVLE LESFQAAAL MEVDIGHL IAREGDCAP FGIELMEVD FYQENYLEY LLGDNQIMP	CIGLISYDGILGDQN EEKIKWEELSVLE FPIIDLESEFQAAALSRK IELMEVDPIGHLYIF LAIAIAREGDCAPEEK QLVFGIHELMEVDIG TQHFHYQENYLEYRQV YDGLLGDNQIMPKAG	183 220 100 166 208 161 249 188						-0.0025 0.0038 0.0036 1.8000 -0.0025 0.0150 0.2800 0.0080			2029 2030 2031 2032 2033 2034 2035 2036

Table XXXaB. GCF DR3a Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
LSYDGLLGD IWEELSVLE LESEFOAAL MEVDPIGHL IAREGDCAP FGIELMEVD FVOENYLEY LLGDNQIMP	CLGLSYDGLLGDQNQI EEKIWEELSVLEVFIE FPDLSEFFQQAAALSRK IELMEVDPIGHLYIF LAIAREGDCAPEEK QLVFCIELMEVDPIG TOHFVQENYLEYRQV YDGGLGDQNQIMPAG	0.0130	0.0027	0.0130			2029 2030 2031 2032 2033 2034 2035 2036

Table 1. Motif Peptides with Binding Data

Figure 2 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
AISRSRKAVME	EFQAAISRSRKAVMEVH						2037
MPLERQRSQHCKP	MPLERQRSQHCKP						2038
IGKGEFPHISY	TLKIGGEFPHISYPL						2019
LHHTLKG	VKVVLHHTLKGEGHII						2040

Table XXb
Mage 3 DR 3b Motif Peptides with Binding Data

Core Sequence	Exemplary Sequence	DR6w19	DR7	DR8w2	DR9	DRw53	SEQ ID NO.
ILGDPKLL AALSRKVAE MPLEQRSQH	EDSILGDPKKLLTQH EFQVQLASRKVAELVII MPLEQRSQHCKP	0.0130	-0.0014	0.0029			2041 2042 2043

TABLE XXI. Population coverage with combined HLA Supertypes

<u>HLA-SUPERTYPES</u>	PHENOTYPIC FREQUENCY					Average
	Caucasian	North American Black	Japanese	Chinese	Hispanic	
<u>a. Individual Supertypes</u>						
A2	45.8	39.0	42.4	45.9	43.0	43.2
A3	37.5	42.1	45.8	52.7	43.1	44.2
B7	38.6	52.7	48.8	35.5	47.1	44.7
A1	47.1	16.1	21.8	14.7	26.3	25.2
A24	23.9	38.9	58.6	40.1	38.3	40.0
B44	43.0	21.2	42.9	39.1	39.0	37.0
B27	28.4	26.1	13.3	13.9	35.3	23.4
B62	12.6	4.8	36.5	25.4	11.1	18.1
B58	10.0	25.1	1.6	9.0	5.9	10.3
<u>b. Combined Supertypes</u>						
A2, A3, B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44, A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, B58	99.9	99.6	100.0	99.8	99.9	99.8

SF 184895 v1

Table XXII. A2 supermotif analogs

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles Crossbound
MAGE3.112	9	KVAELVHFL	69	29	14	168	17	5
MAGE3.112L2	9	KLAELVHFL	20	6.0	5.9	12	400	5
MAGE3.112M2	9	KMAELVHFL	24	6.7	7.7	26	286	5
MAGE3.112L2V9	9	KLAELVHFV	14	13	22	15	73	5
MAGE3.112M2V9	9	KMAELVHFV	26	17	46	39	170	5
MAGE3.220	9	KIWEELSVL	333	391	2381	308	--	3
MAGE3.220L2V9	9	KLWEELSVV	11	165	20	15	--	4

-- indicates binding affinity = 10,000nM.

Table XXIIA A01 Analog Peptides

<u>Peptide</u>	<u>AA</u>	<u>Sequence</u>	<u>Source</u>	<u>A*0101 nM</u>
52.0026	8	ATCLGLSY	MAGE3.179	227.3
52.013	11	VVEVVPISHLY	MAGE2.166	125
52.0132	11	TMNYPLWSQSY	MAGE3.74	301.2
52.0133	11	LMEVDPIGHLY	MAGE3.166	3.3
57.0003	8	VTDLGLSY	MAGE2.179.D3	2.7
57.0029	9	STFSTTINY	MAGE2.69.T2	490.2
57.003	9	MTDLVQENY	MAGE2.247.T2	0.8
57.0031	9	STLPTTMNY	MAGE3.69.T2	58.1
57.0032	9	GTVVGNWQY	MAGE3.137.T2	36.2
57.0033	9	ETDPIGHLY	MAGE3.168.T2	0.7
57.0034	9	ITGGPHISY	MAGE3.293.T2	36.2
57.0119	10	ATSFSTTINY	MAGE2.68.T2	454.5
57.012	10	ASDFSTTINY	MAGE2.68.D3	25
57.0121	10	LTQDLVQENY	MAGE2.246.T2	58.1
57.0122	10	ATSLPTTMNY	MAGE3.68.T2	208.3
57.0123	10	ASDLPTTMNY	MAGE3.68.D3	2.6
57.0124	10	LTDHFVQENY	MAGE3.246.D3	2.3

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Table XXIIIB A03 Analog Peptides

<u>Peptide</u>	<u>AA</u>	<u>Sequence</u>	<u>Source</u>	<u>A*0301 nM</u>	<u>A*1101 nM</u>	<u>A*3101 nM</u>	<u>A*3301 nM</u>	<u>A*6801 nM</u>	<u>A3 XRN</u>
1371.63	9	SVFSTTINK	MAGE2.69.V2K9	20	8.2	3333.3	9666.7	5.7	3
1371.64	9	SVFSTTINR	MAGE2.69.V2R9	57.9	6.3	62.1	87.9	6.7	5
1371.65	9	TVINYTLWR	MAGE2.73.V2	261.9	76.9	720	432.8	14.5	4
1371.66	9	TVINYTLWK	MAGE2.73.V2K9	305.6	96.8	9000	-58000	61.5	3
1371.68	9	LVHFLLLKR	MAGE2/3.116.R9	440	375	236.8	93.5	26.7	5
1371.69	9	YVFPVIFSK	MAGE3.138.V2	24.4	3	2769.2	783.8	1.7	3
1371.7	9	YVFPVIFSR	MAGE3.138.V2R9	35.5	2.6	6	13.2	0.5	5
1371.71	8	SVFAHPRR	MAGE2.237.R8	687.5	1538.5	620.7	580	156.9	1
1371.72	9	AVIETSYVK	MAGE2.277.V2	392.9	62.5	12857.1	-290000	30.8	3
1371.73	9	AVIETSYVR	MAGE2.277.V2R9	36666.7	171.4	128.6	1160	15.4	3
1371.74	9	IVYPLLHER	MAGE2.299.V2	117	375	94.7	32.2	13.8	5
1371.75	9	IVYPLLHEK	MAGE2.299.V2K9	42.3	103.4	857.1	2989.7	42.1	3

Table XXIIC A24 Analog Peptides

<u>Peptide</u>	<u>AA</u>	<u>Sequence</u>	<u>Source</u>	<u>A*2401 nM</u>
52.0072	8	LWGPRALI	MAGE2.272	100
52.0073	8	QYFFPVIF	MAGE3.144	100
52.0078	8	SYPPLHEW	MAGE3.300	285.7
52.0102	10	SYPPLHEWVL	MAGE3.300	20.3
52.0166	11	SFSTTINYTLW	MAGE2.70	428.6
52.0167	11	IFSKASEYLQL	MAGE2.150	126.3
52.017	11	IFSKASSSQL	MAGE3.150	131.9
52.0172	11	IWEELSVLEV	MAGE3.221	461.5
57.006	9	MYPDLESEF	MAGE2.97.Y2	52.2
57.0061	9	KYVELVHFF	MAGE2.112.Y2F9	7.1
57.0062	9	IYSKASEYF	MAGE2.150.Y2F9	14.6
57.0063	9	EYLQLVFGF	MAGE2.156.F9	4
57.0064	9	VYPKTGLLF	MAGE2.195.Y2F9	5.5
57.0065	9	TYPDLESEF	MAGE3.97.Y2	218.2
57.0066	9	NYQYFFPVF	MAGE3.142.Y2F9	3.4
57.0067	9	IYSKASSSF	MAGE3.150.Y2F9	375
57.0068	9	IYPKAGLLF	MAGE3.195.Y2F9	9.2
57.0084	10	SYSTTINYTF	MAGE2.70.Y2F10	14.8
57.0085	10	LYILVTCLGF	MAGE2.175.F10	17.6
57.0086	10	VYPKTGLLIF	MAGE2.195.Y2F10	2.9
57.0087	10	EYLWGPRALF	MAGE2.270.Y2F10	10
57.0088	10	SYVKVLHHTF	MAGE2.282.F10	34.3
57.009	10	NYQYFFPVIF	MAGE3.142.Y2	22.6
57.0092	10	LYIFATCLGF	MAGE3.175.F10	10
57.0093	10	IYPKAGLLIF	MAGE3.195.Y2F10	1.2
57.0095	10	SYPPPLHEWVF	MAGE3.300.F10	5.5

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Table XXIII. Immunogenicity of A2 supermotif peptides

Source	AA	Sequence	A*0201	A*0202	A*0203	A*0206	A*6802	No. A2	CTL	Wild-type	CTL
			nM	nM	nM	nM	Crossbound	Alleles			Tumor
MAGE2.11 ¹	9	KMVELVHFL	9.8	25	17	123	2353	4	1/1		0/1
MAGE2.11 ¹	10	KMVELVHFLL	23	39	127	9.0	2667	4	1/1		0/1
MAGE2.11 ¹	11	KMVELVHFLLL	5.0	45	63	109	7692	4	1/1		0/1
MAGE2.15 ¹	9	KASEYLQLV	152	116	17	185	4878	4	2/4		0/2
MAGE2.15 ¹	10	YLQLVFGIEV	50	165	345	370	9302	4	3/3		1/3
MAGE2.16 ¹	10	LVFGIEVVEV	357	20	43	28	8.0	5	4/4		0/3
MAGE3.11 ¹	9	KVAELVHFL	68	29	14	168	17	5	3/4		3/4
MAGE3.11 ¹	10	KVAELVHFLL	54	36	217	206	11	5	0/1		0/1
MAGE3.15 ¹	11	QLVFGIELMEV	7.9	74	217	185	267	5	3/3		1/3 ²
MAGE3.16 ¹	10	LVFGIELMEV	29	20	7.7	28	14	5	4/4		1/4 ²
MAGE3.19 ¹	11	IMPKAGLLIV	20	226	14	176	-- ³	4	3/4		0/3
MAGE3.22 ¹	9	KIWEELSVL	357	391	2381	308	--	3	3/4		0/3
MAGE3.27	9	FLWGPRALV	31	43	14	336	40	5	4/4		2/4

1) Indicates the number of donors positive over the total number of donors tested.

2) A positive result was seen after the second restim.

3) -- indicates binding affinity =10,000nM.

Table XIV. MHC-peptide binding assays cell lines and radiolabeled ligands.

A. Class I binding assays

Species	Antigen	Allele	Cell line	Radiolabeled peptide	
				Source	Sequence
Human	A1	A*0101	Steinlin	Hu. J chain	YTAVVPLVY
	A2	A*0201	JY	HBV c 18-27	F6->Y
	A2	A*0202	P815 (transfected)	HBV c 18-27	F6->Y
	A2	A*0203	FUN	HBV c 18-27	F6->Y
	A2	A*0206	CLA	HBV c 18-27	F6->Y
	A2	A*0207	721.221 (transfected)	HBV c 18-27	F6->Y
	A3		GM3107	non-natural (A3CON1)	
	A11		BVR	non-natural (A3CON1)	
	A24	A*2402	KAS116	non-natural (A24CON1)	AYIDNYNKF
	A31	A*3101	SPACH	non-natural (A3CON1)	KVFPYALINK
	A33	A*3301	LWAGS	non-natural (A3CON1)	KVFPYALINK
	A28/68	A*6801	C1R	HBV c 141-151	T7->Y
	A28/68	A*6802	AMAI	HBV pol 646-654	C4->A
	B7	B*0702	GM3107	A2 signal seq. 5-13	(L7->Y)
	B8	B*0801	Steinlin	HIV gp 586-593	Y1->F, Q5->Y
	B27	B*2705	LG2	R 60s	FLKDYQLL
	B35	B*3501	C1R, BVR	non-natural (B35CON2)	FRYNGLIHR
	B35	B*3502	TISI	non-natural (B35CON2)	FPRKYAAAF
	B35	B*3503	EHM	non-natural (B35CON2)	FPRKYAAAF
	B44	B*4403	PITOUT	EF-1 G6->Y	AEMGKYSFY
	B51		KAS116	non-natural (B35CON2)	FPRKYAAAF
	B53	B*5301	AMAI	non-natural (B35CON2)	FPRKYAAAF
	B54	B*5401	KT3	non-natural (B35CON2)	FPRKYAAAF
	Cw4	Cw*0401	C1R	non-natural (C4CON1)	QYDDAVYKL
	Cw6	Cw*0602	721.221 transfected	non-natural (C6CON1)	YRHDDGNVL
	Cw7	Cw*0702	721.221 transfected	non-natural (C6CON1)	YRHDDGNVL
Mouse	D ^b		EL4	Adenovirus E1A P7->Y	SGPSNTYPEI
	K ^b		EL4	VSV NP 52-59	RGYVFQGL
	D ^d		P815	HIV-IIIB ENV G4->Y	RGPYRAFVTI
	K ^d		P815	non-natural (KdCON1)	KFNPMKTYI
	L ^d		P815	HBVs 28-39	IPQSLLDSYWTSL

B. Class II binding assays

Species	Antigen	Allele	Cell line	Source		Radiolabeled peptide Sequence
Human	DR1	DRB1*0101	LG2	HA	Y307-319	YPK YVK QNTLKLAT
	DR2	DRB1*1501	L466.1	MBP	88-102Y	VVHFFKNIVTPRTPPYY
	DR2	DRB1*1601	L242.5	non-natural (760.16)		YAAFAAKTAAAFAA
	DR3	DRB1*0301	MAT	MT 65kD	Y3-13	YKTIAFDEEARR
	DR4w4	DRB1*0401	Preiss	non-natural (717.01)		YARFQSQTTLKLQKT
	DR4w10	DRB1*0402	YAR	non-natural (717.10)		YARFQRQTTLKLQAA
	DR4w14	DRB1*0404	BIN 40	non-natural (717.01)		YARFQSQTTLKLQKT
	DR4w15	DRB1*0405	KT3	non-natural (717.01)		YARFQSQTTLKLQKT
	DR7	DRB1*0701	Pitout	Tet. tox.	830-843	QYIKANSKFIGITE
	DR8	DRB1*0802	OLL	Tet. tox.	830-843	QYIKANSKFIGITE
	DR8	DRB1*0803	LUY	Tet. tox.	830-843	QYIKANSKFIGITE
	DR9	DRB1*0901	HID	Tet. tox.	830-843	QYIKANSKFIGITE
	DR11	DRB1*1101	Sweig	Tet. tox.	830-843	QYIKANSKFIGITE
	DR12	DRB1*1201	Herluf	unknown eluted peptide		EALIHQLKINPYVLS
	DR13	DRB1*1302	H0301	GM3107 or L416.3		QYIKANAKFIGITE
	DR51	DRB5*0101	L255.1			PKYVKQNTLKLAT
	DR51	DRB5*0201	MAT			NGQIGNDPNRDIL
	DR52	DRB3*0101	L257.6			YARFQSQTTLKLQKT
	DR53	DRB4*0101	PF	non-natural (717.01)		
	DQ3.1	A1*0301/DQB1*0		non-natural (ROIV)		YAHAAAHAAAHAAAHAA
Mouse	IA ^b		DB27.4	non-natural (ROIV)		YAHAAAHAAAHAAAHAA
	IA ^d		A20	non-natural (ROIV)		YAHAAAHAAAHAAAHAA
	IA ^k		CH-12	HEL 46-61		YNTDGSTDYGILQINSR
	IA ^s		LS102.9	non-natural (ROIV)		YAHAAAHAAAHAAAHAA
	IA ^u		91.7	non-natural (ROIV)		YAHAAAHAAAHAAAHAA
	IE ^d		A20	Lambda repressor 12-26		YLEDARRKKKAIYEKKK
	IE ^k		CH-12	Lambda repressor 12-26		YLEDARRKKKAIYEKKK

Table XXV. Antibodies used in MHC purification.

Monoclonal antibody	Specificity
W6/32	HLA-class I
B123.2	HLA-B and C
IVD12	HLA-DQ
LB3.1	HLA-DR
M1/42	H-2 class I
28-14-8S	H-2 Db and Ld
34-5-8S	H-2 Dd
B8-24-3	H-2 Kb
SF1-1.1.1	H-2 Kd
Y-3	H-2 Kb
10.3.6	H-2 IAk
14.4.4	H-2 IE _d , IE _K
MKD6	H-2 IA _d
Y3JP	H-2 IA _b , IA _s , IA _u

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Table XXVI. Crossbinding data A2 supermotif peptides

Source	AA	Sequence	A*0201 nM	A*0202 nM	A*0203 nM	A*0206 nM	A*6802 nM	No. A2 Alleles	Crossbound
MAGE2.112	9	KMVELVHFL	38	15	9.1	49	364	5	
MAGE2.112	10	KMVELVHFLL	23	39	127	9.0	2667	4	
MAGE2.112	11	KMVELVHFLLL	5.0	45	63	109	7692	4	
MAGE2.153	9	KASEYLQLV	152	116	17	185	4878	4	
MAGE2.157	10	YLQLVFGIEV	50	165	345	370	9302	4	
MAGE2.160	10	LVFGIEVVVEV	357	21	44	29	8.0	5	
MAGE2.220	9	KIWEELSML	167	642	175	29	--	3	
MAGE2.271	9	FLWGPRALI	238	96	137	1542	95	4	
MAGE2.277	10	ALIETSYVKV	500	729	125	1947	3077	2	
MAGE2/3.44	10	TLVEVTLGEV	67	39	4.3	218	33	5	
MAGE3.112	9	KVAELVHFL	68	29	14	168	17	5	
MAGE3.112	10	KVAELVHFLL	54	36	217	206	11	5	
MAGE3.159	11	QLVFGIELMEV	7.9	74	217	185	267	5	
MAGE3.160	10	LVFGIELMEV	29	20	7.7	29	14	5	
MAGE3.174	11	HLYFATCLGL	56	741	769	--	4494	1	
MAGE3.176	9	YIFATCLGL	185	45	37	1028	222	4	
MAGE3.195	11	IMPKAGLLIV	20	226	15	176	--	4	
MAGE3.220	9	KIWEELSVL	333	391	2381	308	--	3	
MAGE3.271	9	FLWGPRALV	31	43	14	336	40	5	

-- indicates binding affinity = 10,000nM.

Table XXVII. Immunogenicity of A2 supermotif peptides

Source	AA	Sequence	A*0201			A*0202			A*0203			A*6802			No. A2 Alleles	CTL Wild-type	CTL Tumor
			nM	nM	nM	nM	nM	nM	nM	nM	nM	Crossbound					
MAGE2.11:	9	KMVELVHFL	9.8	25	17	123	2353	4							1/1	0/1	
MAGE2.11:	10	KMVELVHFLL	23	39	127	9.0	2667	4							1/1	0/1	
MAGE2.11:	11	KMVELVHFLLL	5.0	45	63	109	7692	4							1/1	0/1	
MAGE2.15:	9	KASEYLQLV	152	116	17	185	4878	4							2/4	0/2	
MAGE2.15:	10	YLQLVFGIEV	50	165	345	370	9302	4							3/3	1/3	
MAGE2.16:	10	LVFGIEVVEV	357	20	43	28	8.0	5							4/4	0/3	
MAGE3.11:	9	KVAELVHFL	68	29	14	168	17	5							3/4	3/4	
MAGE3.11:	10	KVAELVHFLL	54	36	217	206	11	5							0/1	0/1	
MAGE3.15:	11	QLVFGIELMEV	7.9	74	217	185	267	5							3/3	1/3 ²	
MAGE3.16:	10	LVFGIELMEV	29	20	7.7	28	14	5							4/4	1/4 ²	
MAGE3.19:	11	IMPKAGLLIV	20	226	14	176	... ³	4							3/4	0/3	
MAGE3.22:	9	KIWEELSVL	357	391	2381	308	--	3							3/4	0/3	
MAGE3.27	9	FLWGPRALV	31	43	14	336	40	5							4/4	2/4	

1) Indicates the number of donors positive over the total number of donors tested.

2) A positive result was seen after the second restim.

3) -- indicates binding affinity =10,000nM.

Table XXII. DR supertype primary binding

Peptide	DR147 Algo Sum	Sequence	Source	DR1 nM	DR4w4 nM	DR7 nM	DR147 Cross- binding
39.0282	2	LGEVPAADSPSPPHS	MAGE2.50	--	--	--	0
39.0283	3	ESEFQAAISRKMVEL	MAGE2.102	4.2	281	49	3
39.0284	2	GIEVVEVVPISHLYI	MAGE2.163	595	6429	278	2
39.0285	2	DGLLGDNQVMPKTGL	MAGE2.187	--	--	--	0
39.0286	2	NQVMPKTGLLIIVLA	MAGE2.193	2632	--	--	0
39.0287	2	KTGLLIIVLAIIAIE	MAGE2.198	417	1216	862	2
39.0288	2	TGLLIIVLAIIAIEG	MAGE2.199	6250	--	--	0
39.0291	2	GLLIIIVLAIIAIEGD	MAGE2.200	500	--	--	1
39.0292	3	LLIIVLAIIAIEGDC	MAGE2.201	581	3750	1923	1
39.0293	2	LIIVLAIIAIEGDCA	MAGE2.202	417	8824	2083	1
39.0294	2	EPHISYPPPLHERALR	MAGE2.296	--	--	--	0
39.0295	3	ALGLVGAQAPATEEQ	MAGE2/3.22	152	--	--	1
39.0296	2	ESEFQAALSRKVAEL	MAGE3.102	2.6	763	34	3
39.0297	2	NWQYFFPVIFSKASS	MAGE3.142	46	409	446	3
39.0298	3	PVIFSKASSSLQLVF	MAGE3.148	98	1875	281	2
39.0299	3	LQLVFGIELMEVDPI	MAGE3.158	200	--	258	2
39.0300	3	GHLYIFATCLGLSYD	MAGE3.173	455	4091	--	1
39.0301	2	DGLLGDNQIMPAGL	MAGE3.187	--	--	--	0
39.0302	2	NQIMPKAGLLIIVLA	MAGE3.193	114	--	--	1
39.0303	2	KAGLLIIVLAIIARE	MAGE3.198	1163	--	--	0
39.0304	2	AGLLIIVLAIAREG	MAGE3.199	1111	--	>9615	0
39.0305	3	LLIIVLAIAREGDC	MAGE3.201	1923	--	--	0
39.0306	2	GPHISYPPPLHEWVLR	MAGE3.296	2273	--	--	0

-- indicates binding affinity =10,000nM.

Table XXIX. DR superfamily crossbinding

Peptide	Sequence	Source	DR1	DR4w4	DR7	DR2w2	DR2w2	DR5w1	DR8w2	Broad Cross- binding (5/8)
			nM	nM	B1 nM	B2 nM	9 nM	1 nM	nM	
39.0283	ESEFQAAISRKMVEL	MAGE2.102	4.2	281	49	147	20	522	741	1581
39.0284	GIEVVEVVPISHLYI	MAGE2.163	595	6429	278	1978	--	49	--	5506
39.0287	KTGLLIVLAIIAE	MAGE2.198	417	1216	862	2460	--	2333	--	--
39.0296	ESEFQAAALSRKVAEL	MAGE3.102	2.6	763	34	29	18	7000	645	1140
39.0297	NWQYFFPVIFSKASS	MAGE3.142	46	409	446	3033	667	--	308	223
39.0298	PVIFSKASSSLQLVF	MAGE3.148	98	1875	281	535	--	146	--	--
39.0299	LQLVFGIELMEVDPI	MAGE3.158	200	--	258	4550	--	8750	--	--

-- indicates binding affinity = 10,000nM.

Table XXX. DR3 binding

Peptide	Sequence	Source	DR3 nM
39.0384	GPRMFPDLESEFQAA	MAGE2.94	3371
39.0387	FPDLESEFQAAISRK	MAGE2.98	--
39.0388	EFQAAISRKMVELVH	MAGE2.104	--
39.0389	QLVFGIEVVVEVPIS	MAGE2.159	--
39.0390	CLGLSYDGLGDNQV	MAGE2.181	2143
39.0391	YDGLLGDNQVMPKTG	MAGE2.186	--
39.0392	LAIIAIEGDCAPEEK	MAGE2.206	--
39.0393	IIAIEGDCAPEEKIW	MAGE2.208	4546
39.0394	EEKIWEELSMLEVFE	MAGE2.218	--
39.0395	RKLLMQDLVQENYLE	MAGE2.243	2000
39.0396	MQDLVQENYLEYRQV	MAGE2.247	1500
39.0397	VKVLHHTLKIGGEPH	MAGE2.284	--
39.0398	TLKIGGEPHISYPPL	MAGE2.290	--
39.0399	FPDLESEFQAALSRK	MAGE3.98	--
39.0400	EFQAALSRKVVAELVH	MAGE3.104	--
39.0401	QLVFGIELMEVDPIG	MAGE3.159	--
39.0402	IELMEVDPIGHLYIF	MAGE3.164	167
39.0403	CLGLSYDGLGDNQI	MAGE3.181	--
39.0404	YDGLLGDNQIMPKAG	MAGE3.186	--
39.0405	LAIIAREGDCAPEEK	MAGE3.206	--
39.0406	EEKIWEELSLEVFE	MAGE3.218	--
39.0407	EDSILGDPKKLLTQH	MAGE3.235	448
39.0408	TQHFVQENYLEYRQV	MAGE3.247	1071

-- indicates binding affinity =10,000nM.

0 9 7 5 2 6 2 6 2 1 2 0 9 6

Table XXXI. HIL Candidates

Peptide	Sequence	Motif	Source	DR1 nM	DR4w4 nM	DR7 nM	DR3 nM	DR2w2 β1 nM	DR6w1 β2 nM	DR5w1 1 nM	DR8w2 nM	DR147 Cross- binding (5/8)	Broad Binding (5/8)	DR3 Binder	
39.0283	ESEFQAAISRKMVVEL	DR sup	MAGE2.102	4.2	281	49	--	147	20	522	741	1581	3	7	0
39.0296	ESEFQAALSRKVVAEL	DR sup	MAGE3.102	2.6	763	34	--	29	18	7000	645	1140	3	6	0
39.0297	NWQYFFPVIFSKASS	DR sup	MAGE3.142	46	409	446	--	3033	667	--	308	223	3	6	0
39.0402	IELMEVDPIGHLYIF	DR3	MAGE3.164	--	>8182	9259	167	1597	--	269	--	3769	0	1	1
39.0407	EDSILGDPKLLTQH	DR3	MAGE3.235	--	>8182	--	448	--	--	269	--	--	0	1	1

-- indicates binding affinity = 10,000nM.